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WITCHES' BROOM OF POTATOES AND TOMATOES [1]

By P. A. YOUNG, *Assistant Botanist and Plant Pathologist*, and H. E. MORRIS, *Associate Botanist and Plant Pathologist, Montana Agricultural Experiment Station* [2]

POTATO WITCHES' BROOM

INTRODUCTION

A study of potato witches' broom was started at the Montana Agricultural Experiment Station in 1915. At that time it was not

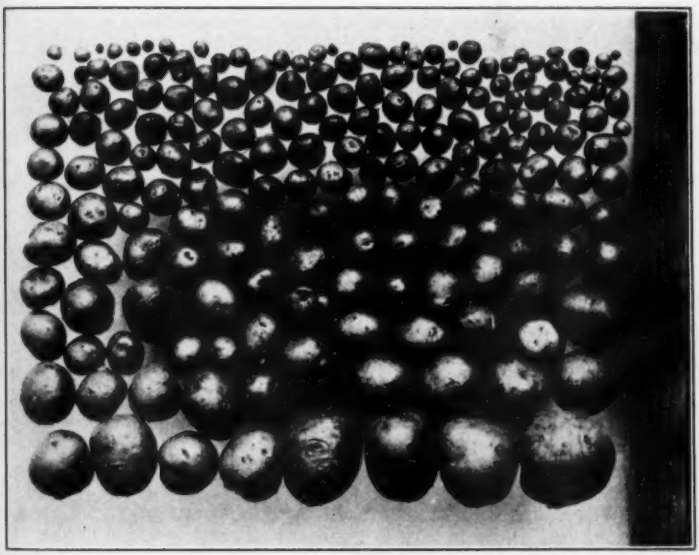

FIG. 1.—Tubers from one potato plant dug September 25, 1915, showing the effect of witches' broom. The 184 tubers cover an area of only 192 sq. cm.

recognized as distinct from the disease caused by Rhizoctonia, and was grown experimentally in connection with the latter disease. However, the photographs and descriptions prepared in 1915 and 1916 show unquestionably that some of the potatoes concerned were affected with witches' broom instead of Rhizoctonia. (Fig. 1.) The

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[2] Thanks are due Prof. D. B. Swingle for criticizing the manuscript.

records show that this virosis [3] was transmitted by the tubers. Observations on witches' broom were made from 1915 to 1920, but were thereafter discontinued because the disease was not very important economically. Increased prevalence led to intensive study of witches' broom from 1925 to 1928.

In 1925, a strain of Jersey Peachblow potato was discarded because 10 per cent of the plants had witches' broom. The next year, in a sample of a stock of Bliss Triumph potatoes submitted for certification, 57 per cent of the 98 hills in the test row showed disease. The tubers used in planting this row weighed from 4 to 8 ounces apiece, and were cut into about 2-ounce seed pieces. The disease recurred in serious amounts in 1927 when a 6-acre field of Bliss Triumph potatoes grown from certified seed at Three Forks, Mont., showed 17 per cent of witches' broom. Many other fields likewise showed increases in witches' broom since 1925, but the amount of the disease in those cases was less than 17 per cent of the crop. In the State of Washington the ravages of witches' broom destroyed a 30-acre field of potatoes, there being 100 per cent infection.[4] Jaczewski (*9*) [5] reports this disease in Russia.

Observations and experiments have not yet revealed the method by which the disease spreads in the field. It occurs in the progeny of potatoes which show no symptoms easily recognizable by men roguing the fields. This virosis is very injurious to potatoes, for affected plants produce very few marketable tubers even when they show only primary symptoms.

SYMPTOMS OF POTATO WITCHES' BROOM

Potato witches' broom was well illustrated and described by Hungerford and Dana (*8*). It was illustrated and briefly described by Whipple (*23*), Coons and Kotila (*3*), Bisby and Tolaas (*2*), McKay (*15*), and Sanford (*19*). Descriptions of witches' broom were also given by Young and Morris,[6] Young (*24*), McLarty (*17*), and Cutler and Sanford (*4*). Spindling sprout is unlike witches' broom in that it is not a single disease due to a single cause, but is just a symptom caused by physiological conditions, leaf roll, or witches' broom.

The primary and secondary symptoms of witches' broom are usually distinct, although they intergrade frequently. The first symptom is an increasingly prominent flavescence [7] in the new leaflets on one or more stems. Marginal flavescence, which consists of light green or yellow margins on green leaves, was observed in 10

[3] "Virosis" is a name for virus disease proposed by Dr. L. R. Jones at Lincoln, Nebr., on Dec. 28, 1925.

[4] UNITED STATES DEPARTMENT OF AGRICULTURE, BUREAU OF PLANT INDUSTRY. WITCHES' BROOM OF POTATO. U. S. Dept. Agr., Bur. Plant Indus. Plant Disease Rptr. 10: 72. 1926. [Mimeographed.]

[5] Reference is made by number (italic) to "Literature cited," p. 853.

[6] YOUNG, P. A., and MORRIS, H. E. POTATO WITCHES' BROOM IS A TRANSMISSIBLE DISEASE. (A PRELIMINARY REPORT.) U. S. Dept. Agr., Bur. Plant Indus. Plant Disease Rptr. 10: 26–28. 1926. [Mimeographed.]

[7] The term "flavescence" is proposed to describe the abnormal, nonnecrotic, hyaline appearance and light green or yellow color that characterize the leaves and young stems of diseased plants, at least during the developmental stages preceding maturity. "Chlorosis" is a broad general term commonly used to describe the light green or yellow color noted in diseased plants, and there seems to be no possibility of limiting its meaning to any particular category, even to the extent of separating it as a nonnecrotic symptom from yellowing. Schultz and Folsom (*20*) give a common definition of chlorosis. The term "flavescence" is useful in describing the particular kind of chlorosis occurring in witches' broom, mosaic mottling, peach yellows, and other diseases in which chlorophyll development is abnormally diminished in the juvenile stages. Another kind of chlorosis is the interveinal chlorosis or yellowing of potato leaf roll. Leaves with normal green color slowly become yellow to greenish hyaline between the veins. This is a slow necrosis preceding death by a few weeks. Young and Morris (*6*) described this interveinal yellowing.

varieties of potatoes affected with this disease. Bright yellow margins were seen in the senescent leaves of one normal potato, but this was unlike the marginal flavescence due to witches' broom. Appel (*1*) mentions a marginal yellowing of potato leaves, but his colored plate shows no symptom closely resembling the marginal flavescence caused by witches' broom.

FIG. 2.—Green Mountain potato grown in the greenhouse, showing the willowy growth, dwarfed leaves, and other primary symptoms of witches' broom transmitted to it by a tuber core from a Bliss Triumph potato. The large leaves were produced before the disease symptoms appeared. × ⅛

PRIMARY SYMPTOMS

The tops of potatoes showing the first symptoms of witches' broom grow rapidly, producing new leaflets that are dwarfed, flavescent, and often rugose. (Figs. 2, 3.) The stems bearing them are also flavescent and have unusually long internodes and enlarged nodes. Such stems are cylindrical rather than quadrangular and winged, and even the leaf petioles have wings of less than usual prominence. The lower leaves are normal on potatoes with primary witches' broom because these developed before the appearance of the disease. (Fig. 2.) Especially in the field the tops of witches'-broom potatoes often become purple, and the flavescent margins of the leaflets frequently become pink or red. Whipple (*23*) illustrated a potato vine with primary witches' broom, although he called it a "yellow top degenerate." As now understood, "yellow top degenerate" appears to consist of rugose mosaic, witches' broom, or a combination of the two diseases.

Many flavescent, spindling axillary branches develop all along the stems and bear tops with typical symptoms. Also, basal branches arise and the plant soon assumes a bushy appearance. (Fig. 2.)

The tops of witches'-broom plants often bloom and produce fruit in abnormal profusion. Late in the growing season the subterranean tubers sprout and send up very many spindling little stems with dwarfed leaves around the base of the main stem. These tubers sometimes proliferate and thus produce chains of a few small tubers. Many little aerial tubers with leafy eyes commonly develop on the main stems. Plants showing primary symptoms usually bear 25 to 200 very small subterranean tubers. (Fig. 1.) Besides these, they

generally bear 1 to 3 average-size tubers that developed before the disease symptoms appeared.

Frost usually kills the plants in the field near this stage in the progressive appearance of the symptoms. Their continued development was studied in the greenhouse in plants manifesting primary symptoms as the result of stem grafts and tuber-core grafts. The secondary symptoms appear and gradually replace the primary symptoms. Many of the primary and secondary symptoms are identical and often intergrade.

The primary symptoms of witches' broom are the same in the greenhouse and the field, except that greenhouse plants frequently become

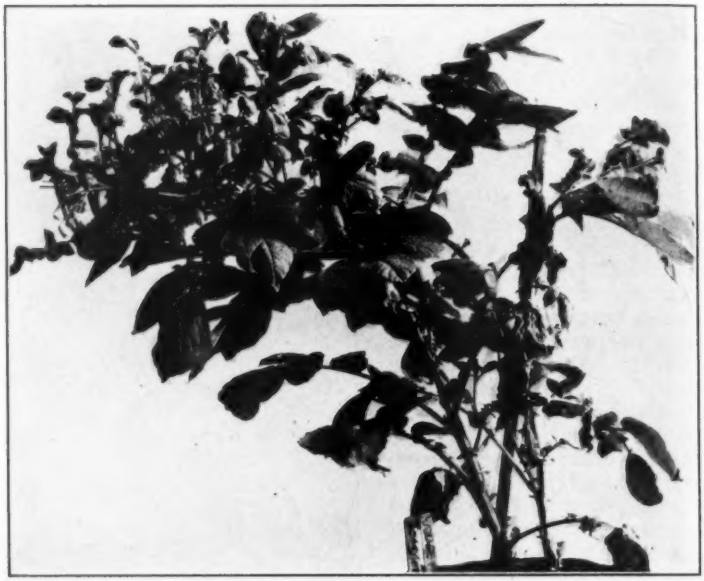


FIG. 3.—Bliss Triumph potato showing primary symptoms of witches' broom transmitted to it by a tuber core from a Jersey Peachblow potato tuber. The top shows many spindling branches with dwarfed, flavescent leaves. The base bears spindling sprouts. × 1/7

very tall and willowy, with narrow vines that are often 0.8 to 1.2 meters tall, while field plants are broad and bushy, or small and upright, depending upon the severity of the disease. (Figs. 2, 3.) The uprightness may be due to mosaic in combination with witches' broom.

An unusual type of floral development occurred in the greenhouse in 1926. Aerial tubers appeared on the old flower trusses of two Bliss Triumph potatoes, and new flowers grew from the eyes of the tubers. (Fig. 4.) One of these plants showed primary symptoms and the other secondary symptoms of witches' broom. Apparently these tubers on old flower stalks possessed the genetic ability to produce flowers because of their position. Large Bliss Triumph plants are ornamental with their yellow to purple tops bearing flowers, red aerial tubers, and filamentous stems. The colors are often brilliant.

Secondary Symptoms

In the further development of a witches'-broom plant, the basal sprouts grow slowly while the main stems grow rapidly. The old leaflets produced while the plant was normal gradually die and are replaced by the dwarfed, chlorotic, often simple leaves produced by the spindling tops and branches. The main stems finally die, completing the transformation of a large, normal potato plant into a dwarfed plant with secondary symptoms. This illustrates the intergradation of the primary and secondary symptoms. (Fig. 2.) While large plants with witches' broom usually bear numerous tubers, plants extremely dwarfed by this disease often produce very few.

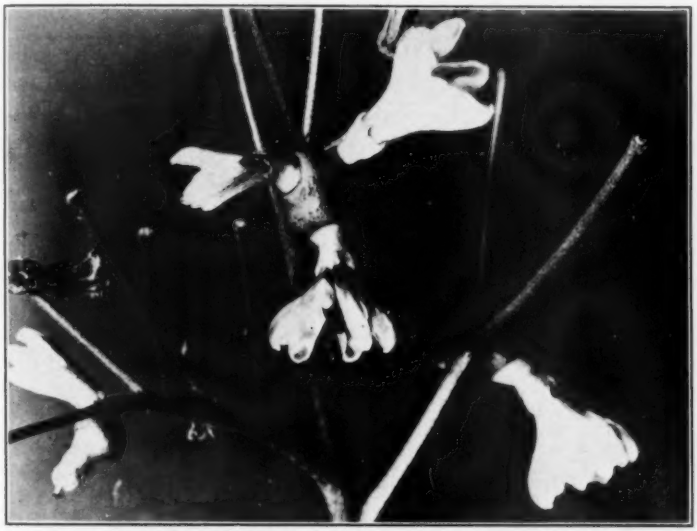


FIG. 4.—Flowers produced by the eyes of aerial tubers borne on an old flower truss. Severe witches' broom was transmitted to this Bliss Triumph potato plant by a tuber core from a diseased Jersey Peachblow potato. × 1⅓

Pubescent, usually leafless, filamentous branches one-fourth to 2 mm. in diameter and one-half to 10 cm. long often appear at many of the nodes of the stem. (Fig. 5.) While this symptom is frequently absent on witches'-broom plants, especially in the field, it is very useful in diagnosis when it occurs. These slender, even threadlike aerial stems are often branched and bear terminal tubers and simple leaves. Sometimes the tips of the filamentous aerial stems grow into the surface of the soil and produce terminal tubers there. Some large plants in the greenhouse bore a hundred or more filamentous stems. Witches'-broom plants in the field, particularly those in cages, often bear such stems. The filamentous stem shown in Figure 5 is unusually elaborate.

On normal Bliss Triumph potatoes in the greenhouse were seen 25 examples of an unusual structure comparable to these filamentous stems. These potatoes bore basal, plagiotropic, hyaline, pubescent,

leafless, apparently functionless stems (or aerial stolons) which were 2 to 12 cm. long and one-half cm. thick. They did not develop further, and so were quite unlike the filamentous stems on diseased potatoes.

While plants that grow from tubers with witches' broom usually display only secondary symptoms, primary symptoms may occur in mild cases of the disease. This was exhibited in the field in 1927 when Bliss Triumph selection No. 115 produced large vines with large compound leaves, even though the plants in the two preceding generations showed secondary symptoms of witches' broom. Very little flavescence occurred in the tops of the vines during most of the season, so the disease was readily detected only by examining the bases of the plants. Each had about 50 stems with some fila-

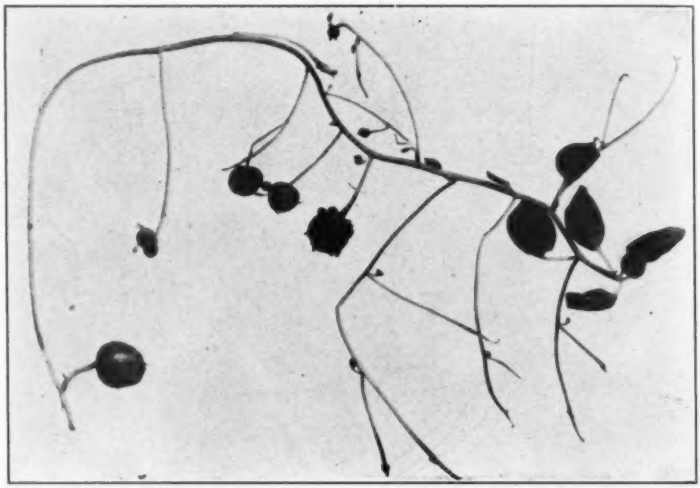


FIG. 5.—Elaborate filamentous aerial stems of a Bliss Triumph potato showing secondary symptoms of witches' broom. The simple leaves are terminal. $\times \frac{1}{12}$

mentous stems and aerial tubers. These plants had the disease in an unusually mild form, yet they produced no tubers of marketable size. The other potatoes of different parentage showed the disease in more severe form with fewer primary symptoms. Although the strains of potatoes selected for study of witches' broom had the disease in widely different degrees of severity, there is not sufficient evidence to conclude that more than one virus was involved.

Sprouts from tubers produced by witches'-broom plants show symptoms of the disease soon after they appear, for each seed piece sends out 3 to 50 spindling little stems with dwarfed and often simple leaves. (Figs. 6, 7.) This spindling sprout is a characteristic symptom at this stage of the disease. These sprouts usually produce field plants with many dwarfed, spindling, partly procumbent stems 12 to 30 cm. tall. This prostrate form is now known to be the most common type of witches' broom, and has been observed in Montana nearly every year since 1915. (Fig. 6.) Hills of this type were

secured by planting two aerial tubers from the top of one Jersey Peachblow plant. Some of the witches'-broom potatoes in the greenhouse exhibited part of the following unusual symptoms. Some had green vines that were abnormal only in that they were

FIG. 6.—A young Bliss Triumph potato plant with witches' broom, showing numerous spindling stems with small leaves. × ½

very spindling and dwarfed. Usually the stems were numerous and dwarfing was extreme. Some potted plants were only 5 to 15 cm. tall at the age of 5 months; most of their leaves were simple. The stems on the smallest of these plants were only one-half to 1 mm. in

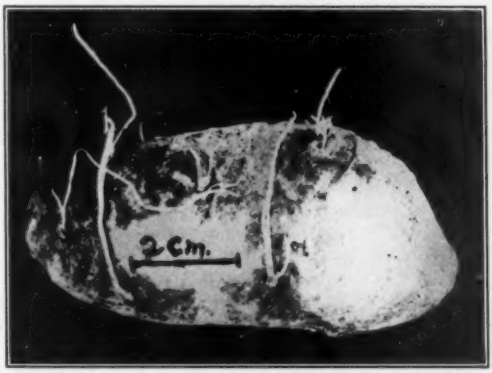


FIG. 7.—Spindling sprouts on a Russet Burbank potato tuber affected with witches' broom. The virus of this disease was transmitted to its parent by an inarch graft with a witches'-broom tomato vine

diameter, and the densely clustered, entirely simple leaves were only 1 to 4 mm. wide. (Pl. 1, F.) On some old witches'-broom potatoes many of the aerial tubers produced 5 to 15 spindling little stems apiece, so that the main stems were adorned with rosettes of little stems. (Fig. 8.) Some senescent vines produced new branches in their tops.

The lower leaves of old witches'-broom plants were sometimes rolled and brittle, suggesting leaf roll. However, they had none of the other symptoms of leaf roll.

Crinkle mosaic often occurs in combination with witches' broom. The symptoms of each disease are usually visible at the same time. In inoculations with both the mosaic and the witches'-broom viruses, the symptoms of mosaic appear first. New leaves produced later which show symptoms of witches' broom often are not mottled.

Tubers produced by witches'-broom plants have a short dormancy period or none, and senescence occurs later in diseased than in normal potatoes. With good care a witches'-broom plant may appear to remain alive for a year or more, as the old stems successively die and

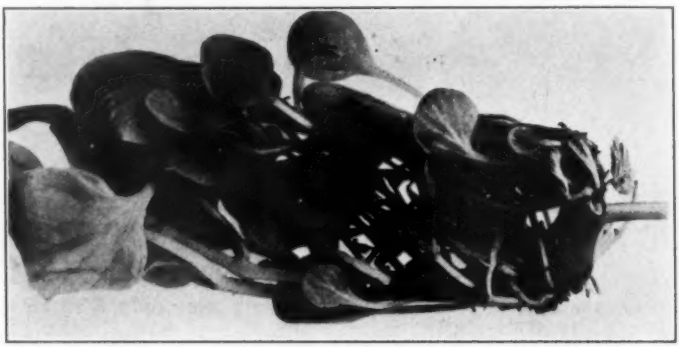


FIG. 8.—Bliss Triumph potato with severe witches' broom, showing an aerial tuber bearing a rosette of many spindling stems and simple little leaves. × 1⅗

are replaced by new sprouts which arise from tubers in or near the surface of the soil.

METHODS OF TRANSMITTING WITCHES' BROOM

General Methods

Seed tubers were cut into 2 to 8 pieces, 1 or 2 of which were saved for controls. The cutting knives and nickel-plated cork borers were disinfected with 5 per cent formaldehyde and then rinsed with water before changing to different scion and stock tubers. The hands were disinfected frequently with laundry soap or a 4 per cent formaldehyde solution. The table was washed with 1 per cent Uspulun before fresh groups of cut seed pieces were laid upon it. Hence, there was little danger of unintentional transference of the witches'-broom virus, especially since it is difficult to transmit. The greenhouse was frequently fumigated with HCN and sprayed with strong nicotine sulphate to control aphids and other insects.

Core Grafts

The method of grafting tubers was essentially the same as that described by Murphy and M'Kay (*18*) and Goss (*6*). From tubers produced by witches'-broom potatoes, cores 2 to 4 cm. long and 0.5 to 1 cm. in diameter were removed. The small size of the diseased tubers did not permit the use of larger cores. When possible, holes

in the stock seed pieces were made parallel to the vascular rings with cork borers one or two sizes smaller than the ones used in removing the scion cores. The bark was cut from the ends of the scion cores, so that these cores from the witches'-broom tubers certainly bore no eyes. After the insertion of the cores the controls and inoculated seed pieces were usually disinfected with 10 per cent Semesan Bel suspension to lessen subsequent rotting. The seed pieces were then planted in a sand bed. Sometimes seed pieces were kept in a damp chamber for a day after cutting to aid in callusing, but rotting was not diminished by this method. Potatoes were transplanted to 15 or 20 cm. pots as soon as the sprouts were 2 to 6 cm. long. The serial numbers written in India ink on the seed pieces usually were readable at the time of transplanting.

Stem Grafts

Three methods of grafting herbaceous stems were used: (1) Cleft grafts were made by inserting scions into clefts made in the stocks; (2) slips (scions) were inserted into slits made in the stems of the stocks; (3) inarch grafts were made by slicing off the cortical layers on one side of each of two stems and binding the cut surfaces together while the roots of both plants remained undisturbed in the soil. All grafts were tightly wrapped with string and painted with hot grafting wax. The grafting wax, preferably just hot enough to be liquid, did not injure the stems of the solanaceous plants used. The drops of grafting wax that fell on the leaves slowly caused unimportant leaf spots. This is a rapid method of protecting cut surfaces from injury due to evaporation. High humidity was maintained in the room only while the grafts were being made. Grafts were not unwrapped for one to two months, so growth kept the cut surfaces in close contact. The resulting constrictions of the stems did not appear to be injurious.

Leaf-Mutilation Inoculations

The method of making leaf-mutilation inoculations was similar to that used by Schultz and Folsom (*20*). Stems and leaves of witches'-broom potato plants were ground in a sterile food grinder and placed in sterile dishes. Care was taken to prevent contamination of the inoculum from the possible presence of a virus in inoculated plants. Each inoculation was made by placing some of the freshly macerated inoculum on a leaf and pressing it against the leaf until the latter was ruptured. Usually 20 of these inoculations were made on each plant. Plants were 8 to 20 cm. tall and growing vigorously at the time of inoculation. In many cases the plants were reinoculated two or three times at intervals of three to seven days. Plants were usually kept damp for 10 to 20 hours after inoculation by spraying water on the steam pipes, walls, and floor more often and more abundantly than usual.

EXPERIMENTS IN TRANSMISSION OF WITCHES' BROOM FROM DISEASED TO HEALTHY POTATOES

Witches' broom was transmitted by tuber-core grafts to 59 potato plants of 11 varieties. (Fig. 9.) The incubation periods varied from 29 to 114 days.

The symptom of marginal flavescence was seen in all the affected varieties except Russet Burbank. In other experiments Smooth Burbank potatoes showed this symptom. Similar core grafts failed to transmit witches' broom to Norwegian Yam, Early Michigan, Early Rose, Producer, and Up-to-date potatoes. However, there were too few inoculations of these varieties to indicate immunity.

FIG. 9.—Chart showing the transmission of potato witches' broom from 4 selections of Bliss Triumph and 4 selections of Jersey Peachblow potatoes having the disease to 11 varieties of potatoes. Solid lines connecting circles show that infection occurred; broken lines show that infection did not occur. The numbers at the top of the small circles refer to the numbers of healthy controls from the same tubers as those inoculated. In the fractions the denominators represent the number of inoculations and the numerators represent the number of infections. All the inoculations were made by means of core grafts except the three marked "S. G." meaning stem grafts. Twelve of the core-grafted plants, including the four marked "field," were grown in the field. All the others were grown in the greenhouse

Of the 142 seed pieces core grafted with scions from Bliss Triumph and Jersey Peachblow potatoes containing the witches'-broom virus, 42 per cent developed plants with unmistakable symptoms of the disease. These graft inoculations were made in three series during 15 months. Cutting off the tips of the potato stems before they became senescent stimulated new growth which showed the disease symptoms. This method hastened the appearance of symptoms in inoculated plants. In two cases the disease symptoms did not appear except in the progeny of the inoculated plants. Some pre-

liminary studies on transmission were reported by Young and Morris [8] and Young (*24*).

Control seed pieces were cut from the same tubers as those furnishing the core-grafted seed pieces. None of the resulting 46 control plants growing near the inoculated plants in the greenhouse developed symptoms of witches' broom.

To study primary symptoms in the field, 30 of the core-grafted seed pieces, with their controls were planted in the field in 1927. Here, the primary symptoms of witches' broom appeared clearly in 4 of the inoculated plants of each of the following varieties: Bliss Triumph, Idaho Rural, and Irish Cobbler. Five of the other 18 core-grafted plants developed questionable symptoms of witches' broom. The 14 field controls remained healthy.

In another experiment 45 normal seed pieces of the Sharples strain of Bliss Triumph potatoes were core grafted with scions from healthy tubers of this variety in an attempt to detect differences between the early and late maturing forms. Cores from the tubers of the early maturing strain were placed in the stock seed pieces of the late maturing strain and vice versa. These plugged seed pieces, and 45 controls from the same tubers, produced plants which grew in the greenhouse for 84 days. None of them developed symptoms of viroses. Besides these control grafts, 4 Bliss Triumph seed pieces were core grafted with scions from healthy Irish Cobbler and Russet Burbank tubers, and 3 Russet Burbank and 2 Irish Cobbler seed pieces were core grafted with scions from healthy Bliss Triumph tubers. None of the seed pieces or the controls from the same tubers developed plants with disease symptoms. These served as checks on the grafts containing diseased scions.

About 10,000 potato plants selected for freedom from witches' broom were closely observed while they developed to maturity in the greenhouse and the field. The parents of these plants had shown no evidence of witches' broom. Only one of these progeny plants developed witches'-broom symptoms, and it probably represented late-season infection of the parent in the field. In contrast to this, hundreds of tubers from witches'-broom potatoes were planted in the field and in the greenhouse, and all of them showed secondary symptoms of the disease, proving that tuber transmission is a regular occurrence.

Vines of 17 normal Bliss Triumph and Perfect Peachblow potatoes were grafted with stem scions from witches'-broom potatoes. Two were inarch grafts and the others were slip grafts. One plant of each variety developed witches'-broom symptoms; one was an inarch graft and the other was a slip graft. Slip grafts were inefficient in transmitting witches' broom under the conditions prevailing, probably because the slips died too soon. Güssow (*7*) transferred mosaic by inarch grafting.

One normal Russet Burbank and four normal Bliss Triumph potatoes were inarch grafted on witches'-broom tomato plants. The tomatoes had acquired the disease through stem grafts with witches'-broom potatoes. One potato of each variety developed vine and tuber symptoms of witches' broom, showing the transference of the virus back to potatoes. (Fig. 7.) In six inarch grafts of

[8] YOUNG, P. A., and MORRIS, H. E. Op. cit.

healthy Bonny Best tomatoes on Russet Burbank potatoes free from witches' broom, none of the plants were affected by the grafts.

By leaf-mutilation inoculations 71 Bliss Triumph, Russet Burbank, and Irish Cobbler potato plants 12 to 30 cm. tall were inoculated with the witches'-broom virus. These experiments were conducted in the greenhouse in three series during two years. None of the inoculated plants or controls developed witches'-broom symptoms. Tubers from 36 of these inoculated plants were grown later in the greenhouse, but none of them developed witches'-broom symptoms. Although rugose mosaic was transmitted frequently by leaf-mutilation inoculations made at the same time, this method appears to be ineffective in transmitting witches' broom.

Eighteen normal potato plants were grown in pots with plants having secondary symptoms of witches' broom, but none of the normal plants became diseased. No evidence of transmission of witches' broom by soil or by root contact was seen by the authors.

Mealy bugs (Coccidae) were colonized on potato vines with severe witches' broom, and then transferred to four healthy Bliss Triumph potato plants and two healthy Earliana tomato plants. No evidence of disease transmission was seen.

A common, rather omnivorous species of greenhouse aphid was colonized for 24 days on potato vines which showed secondary symptoms of witches' broom. Many of the aphids were then allowed to feed for 34 days on the sprouts of two Irish Cobbler potato tubers which were laid under the vines of the diseased plant. The tubers were then planted, and progeny from one of the resulting plants was grown later in the greenhouse. These Irish Cobbler plants developed no symptoms of disease. Nine other attempts to transmit the disease with aphids failed.

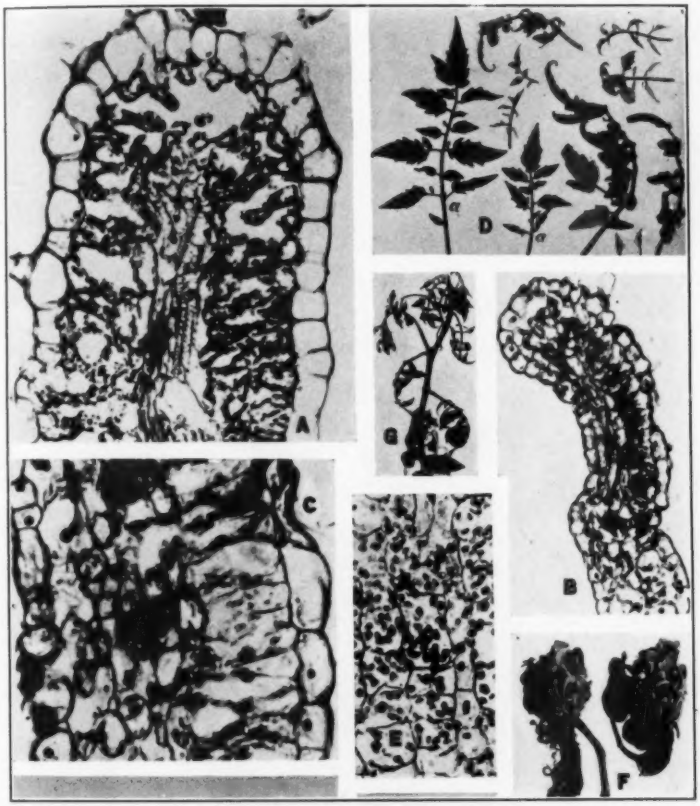
In the field in 1925 and 1926, normal Bliss Triumph potato plants in the rows grew beside others severely affected with witches' broom. Since aphids were abundant on both the healthy and diseased plants late in the season, tubers from eight of the normal plants were planted in the greenhouse at different times. All the resulting plants remained healthy. These experiments in the field and in the greenhouse were too limited to prove that transmission by aphids does not occur. Dana (*5*) reported some indications that green aphids transmit the disease.

The leaves and stems of potato witches'-broom plants were ground in a sterile food grinder. This inoculum was smeared on the freshly cut surfaces of 4 Russet Burbank and Irish Cobbler seed pieces. The remaining inoculum was then diluted with 100 parts of distilled water, and 8 freshly cut seed pieces of Bliss Triumph, Rural New Yorker, Irish Cobbler, and Russet Burbank potatoes were soaked in the liquid for 30 minutes to 2 hours. The potato vines grown from these 12 seed pieces remained healthy, so these methods of inoculation appear to be ineffective.

TOMATO WITCHES' BROOM

SYMPTOMS OF TOMATO WITCHES' BROOM

The symptoms of this disease were seen originally in some tomato stems inarch grafted on potato stems having witches' broom. The first symptom was marginal flavescence in the new leaflets, which is a char-



A.—Photomicrograph of a cross section of a normal Bonny Best tomato leaf showing the numerous chloroplasts and long palisade cells. × 195

B.—Photomicrograph of a cross section of the flavescent margin of a Bonny Best tomato leaf with witches' broom transmitted from a Green Mountain potato. It shows abnormally short palisade cells and relatively few chloroplasts in the chlorenchyma cells. × 176

C.—Photomicrograph of a cross section of a completely flavescent leaf of a Bonny Best tomat plant showing the scarcity of chloroplasts, and the dwarfed palisade cells. × 312

D.—The two leaves marked *a* were cut from a normal Earliana tomato plant. The other leaves were cut from an Earliana tomato plant with witches' broom transmitted to it from a Perfect Peachblow potato. The diseased leaves show extreme flavescence, curling, dwarfing, and in three figures, absence of leaf blades. × ½

E.—Photomicrograph of a longitudinal section of the stem of a Jersey Peachblow potato with secondary symptoms of witches' broom. It shows minute subspherical, nonstriate starch grains in the pith. × 162

F.—Jersey Peachblow potato plant with only flavescent, simple leaves 1 to 4 mm. wide, and stems no longer than 7 cm. at the age of 5 months. These are extreme symptoms of witches' broom. × 1

G.—Earliana tomato showing the symptoms of witches' broom transmitted to it through an inarch graft with a Russet Burbank potato having the disease. The leaves are curled, dwarfed, and flavescent. × ⅙

acteristic symptom of witches' broom. The terminal leaflets usually turned light yellow, pale green, hyaline, or purple. The leaflets were much dwarfed and often rugose. Many of them had very narrow leaf blades, or none at all. (Figs. 10, 11, and pl. 1, D, G.) The leaflets and rhachises were prominently downward curled, but none were upward rolled. In the first series of grafts the Earliana toma-

FIG. 10.—Flavescent Earliana tomato plant, showing witches' broom transmitted to it by inarch grafting on the stem of a diseased Perfect Peachblow potato. Note the bladeless leaflets and dead ends of compound leaves. This plant grew from a small cutting taken from a witches'-broom tomato plant. × 1/7

toes with witches' broom bloomed profusely and produced many small insipid tomatoes containing relatively few seeds. Only a small percentage of these seeds were of normal size and viability. Tomatoes in the later series bore very few fruits. The flowers were normal in appearance and development, except that some were abnormally light in color. Some of these symptoms have been described by Young (*24*).

In one tomato 1.5 meters tall the symptoms appeared in some branches near the base of the plant within 72 days after grafting,

and did not appear in the top of the plant until 44 days later. Witches'-broom symptoms in the tops of tomatoes were not caused by the constrictions in the grafted stems due to long, tight wrapping, as the same symptoms appeared early in branches attached below the grafts in some stems. The witches'-broom potato stems in the grafts lived until symptoms appeared in many of the tomatoes; some lived longer. A few of the potato stems were cut off below the grafts and then grew as scions on the tomatoes.

Senescence was not much delayed in witches'-broom tomato plants. The normal, large, old leaves, that developed before the appearance of the disease symptoms, die, leaving only the flavescent, curled, nearly bladeless leaves on the stems. (Fig. 10.) The

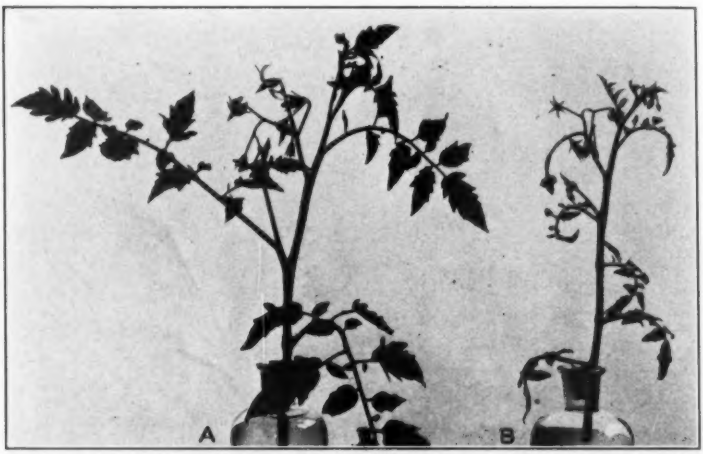


FIG. 11.—A, top of a normal Earliana tomato plant; B, flavescent top of an Earliana tomato plant showing severe symptoms of witches' broom transmitted to it by inarch grafting with a diseased Russet Burbank potato. × ¼

senescent stems turned yellow and became hollow, tough, and partly dry. Normal tomato stems were not hollow.

In this senescent and diseased condition some of the witches'-broom tomatoes lived from one to two months before dying. Slender, flavescent, dwarfed branches grew from the axils of many old leaves. The ends of most of the chlorotic, compound leaves died, leaving only the basal leaflets alive.

To test the persistence of the disease, many cuttings from old witches'-broom tomatoes were planted in sand. Most of them died without growing much, which indicated that they originally contained too little stored food. Normal tomatoes were easily propagated by cuttings. A few of the diseased cuttings grew well and continued to exhibit characteristic symptoms of witches' broom. The one shown in Figure 10 grew for 10 weeks.

WITCHES' BROOM COMPARED WITH OTHER VIRUS DISEASES OF TOMATO

A comparison of witches' broom with other tomato viroses leads to the conclusion that witches' broom is probably a new disease. Since

the virus came from witches'-broom potatoes, the disease is called tomato witches' broom. It differs conspicuously from tomato yellows which causes upward rolling of the leaves, and does not regularly cause flavescent leaf margins and extremely dwarfed leaflets without leaf blades. Witches' broom is unlike mild mosaic or the mosaic transmitted to tomato from potato rugose mosaic. It does not resemble linear (shoe string) or crinkle mosaic in that the leaves are not mottled or long and slender, the flowers are not abnormal, and the tops do not have a fringed appearance. There is only slight similarity between witches' broom and fernleaf. These other viroses of tomato were described by McKay (*13*, *14*), McKay and Dykstra (*16*), Shapovalov (*21*), Weber and Ramsey (*22*), Johnson (*10*), and Kraybill and Eckerson (*11*).

EXPERIMENTS IN TRANSMISSION OF WITCHES' BROOM FROM POTATOES TO TOMATOES

The transmission of the witches'-broom virus between potatoes and tomatoes are shown in Table 1. Thirty-eight ungrafted Bonny Best and Earliana control tomatoes, 28 of which were in the pots with the grafted tomatoes, remained free from witches' broom while they were under observation for two to four months. Besides these, all four plants in the two sets of normal Bonny Best tomatoes grafted together did not show witches' broom while they were watched for 107 days. About one-fourth of the grafted and control tomatoes showed traces of very mild mosaic, but the mottling did not obscure the witches'-broom symptoms. These grafts were made in two series in the greenhouse in 1927.

TABLE 1.—*Transmission of witches' broom involving tomatoes*

Variety with witches'-broom disease	Variety grafted upon	Number of inoculations	Number of inoculations causing disease	Length of incubation period
Perfect Peachblow potato	Earliana tomato	5	3	53 days.
Green Mountain potato	do	1	1	49 days.
Do	Bonny Best tomato	11	6	35 to 82 days.
Colorado Pearl potato	Earliana tomato	1	0	
Bliss Triumph potato	do	7	3	54 to 72 days.
Rural New Yorker potato	do	5	1	53 days.
Blue Victor potato	do	2	1	Do.
Russet Burbank potato	do	1	1	Do.
Six Weeks potato	Bonny Best tomato	1	1	35 days.
Earliana tomato	Bliss Triumph potato	4	1	56 days.
Do	Russet Burbank potato	1	1	63 days.
Do	Bonny Best tomato	11	5	35 to 84 days.

EXPERIMENTS IN SEED TRANSMISSION OF WITCHES' BROOM

In trying to determine the possibility of transmitting the witches'-broom virus through seeds, Earliana tomato plants were grown from seed produced by a tomato with this disease. Sixteen of these plants were kept under observation for 174 days and 19 others for 82 days. Four became abnormal within 27 days and 11 became abnormal later; 6 remained abnormal. The abnormalities consisted of yellowed, rugose lower leaves with necrotic spots, unusually long, twisted teeth on the leaflets, and marginal flavescence. These plants developed

none of the severe symptoms of tomato witches' broom, so these probably were not symptoms of this disease. Although nematodes on tomato roots may cause a chlorosis of the top leaves, none were present on the roots of these plants. The condition caused by nematodes is readily distinguishable from that caused by witches' broom in tomatoes.

In a later series of experiments, 45 Earliana tomato plants were grown from the seed of 6 tomato witches'-broom plants. They showed no symptoms of witches' broom within five months. This fact leads to the conclusion that tomato witches' broom is not transmitted through seeds.

HISTOLOGICAL SYMPTOMS OF WITCHES' BROOM

POTATOES

Mitosis was studied in the buds of four varieties of potatoes showing severe symptoms of witches' broom, and in normal potatoes. The nuclei in the resting and mitotic stages appeared to be the same in the normal and in the witches'-broom potatoes. No abnormal cytological structures were seen.

Iodine was used in an attempt to discover a quick method of identifying viroses. It was mixed with drops of juice expressed from stems, and also painted on stems from which some of the cortical tissues had been removed. Abundant starch was shown by quick blackening. While the stems of witches'-broom potatoes usually contained more starch than normal stems, some normal stems contained abundant stored starch, and some stems of witches'-broom potatoes contained very little starch, so iodine was not very reliable in diagnosis.

Much of the starch seen in the stems was different from tuber starch in that the stem-starch grains were nearly spherical and showed a few lines radiating from the clear centers, rather than being subelliptical with concentric, eccentric striae like tuber starch. (Pl. 1, E.) Grains resembling those of tuber starch were sometimes seen in potato stems.

TOMATOES

Sections of normal and witches'-broom tomato leaves exhibited clear differences between normal and flavescent regions. Normal tomato leaves were 200–325 microns in diameter, with palisade cells 40–70 microns long. The chlorenchyma cells contained numerous chloroplasts. (Pl. 1, A.) In sharp contrast to the normal leaves were the entirely flavescent leaves of diseased plants. These were only 95–200 microns thick, with palisade cells only 25–40 microns long. The region of spongy parenchyma was thin. Very few chloroplasts were seen, which clearly explains the hyaline yellow color of the leaves. (Pl. 1, C.)

Sections of leaves showing marginal flavescence revealed distinct morphological modifications. (Pl. 1, B.) The leaves were only 70–150 microns thick. The palisade cells of the interior of the leaf were 40–50 microns long, but at the margin of the leaves they were only 17–30 microns long. Furthermore, most of these marginal palisade cells were circular to broadly triangular in outline. Abnormally few chloroplasts were present, particularly in the marginal cells, which explains the marginal flavescent color. Intercellular spaces were

abnormally numerous in the flavescent leaves. In the normal leaves the palisade cells at the margin were the same length as those in the interior of the leaves. These sections of tomato leaves showed a clear morphological basis for marginal flavescence. Perhaps because only old material was available, sections of normal and witches'-broom potato leaves did not show such differences.

DISCUSSION

Witches' broom is one of the unmottled viroses that severely injure affected plants. Potatoes attacked by it produce few or no marketable tubers when the tops show prominent symptoms. Tomatoes attacked by it produce no fruit, or poorly flavored small fruits of no value.

Three symptoms are common in witches' broom of potato and tomato: Extreme leaf dwarfing, marginal flavescence of the leaves, and abnormally numerous axillary branches. The disease in these hosts is known to have the same cause, for the virus was directly transmitted from witches'-broom potatoes to tomatoes, and then returned to potatoes in which it produced characteristic symptoms.

Controlled transmissions have been accomplished only by keeping diseased and healthy tissues in close contact for a few weeks. All attempts to transmit the virus by leaf-mutilation inoculations and through the freshly cut surfaces of seed pieces failed. This virus seems to be like the virus of peach yellows in that it is difficult to transmit.

The witches'-broom virus selected in eight hills of potatoes in 1925 was transmitted many times. In one case the virus from a Bliss Triumph potato was transmitted by a tuber plug to a Russet Burbank potato, from which it was transmitted later by an inarch graft to an Earliana tomato. By a stem graft the virus was returned to Bliss Triumph and Russet Burbank potatoes. This exemplifies the way in which the successive transfers of the virus occurred.

Symptoms due to other causes sometimes suggest potato witches' broom. The abnormally numerous axillary branches sometimes borne by potatoes with severe spindle tuber are normally green and not spindling; so they are unlike the axillary branches of witches'-broom potatoes.

Spindling sprout is a characteristic symptom of witches' broom. Tubers from leaf-roll potatoes sometimes produce this symptom too, but the subsequent development of the sprouts is very different in witches' broom. Physiological disturbances sometimes cause spindling sprouts to appear. Whipple (*23*) secured some evidence of this phenomenon. It was observed by the present authors that rotting of seed pieces often caused the resulting sprouts to be very spindling. Hence spindling sprouts seem to be a symptom rather than a separate disease.

The aerial tubers caused by *Corticium vagum* usually are larger than those caused by witches' broom. This is the only important symptom common to both diseases.

The primary symptoms of leaf roll in potato tops may resemble witches' broom for only a short time. Combinations of the two diseases are recognizable.

Richards, Blood, and Linford[9] described a new disease in Utah which has some symptoms that closely resemble those of potato witches' broom. In private conversation, Richards described symptoms of the new disease which clearly distinguish it from witches' broom.

Kunkel (*12*) states that aster yellows is distinct from potato witches' broom.

SUMMARY

Potato witches' broom was discovered in Montana in 1915.

It is so destructive that the percentage of loss is usually the same as the percentage of visible infestation.

The primary symptoms of potato witches' broom are: Dwarfed, flavescent top leaves, often with yellow margins; upper parts of stems cylindrical, with swollen nodes; rapid elongation of stems; profuse branching of the tops; aerial tubers; basal sprouts from tubers; and numerous small subterranean tubers. These symptoms intergrade with the secondary symptoms.

The main secondary symptoms are: Flavescence and purpling of the tops; marginal flavescence and dwarfing of the leaflets; simple leaves; numerous, spindling axillary branches; slender, cylindrical stems with enlarged nodes; filamentous stems; spindling sprouts from tubers or the base of the stem; aerial tubers; and numerous, very small subterranean tubers.

The disease is tuber perpetuated, but the exact means of dissemination in the field is unknown.

Tubers from witches'-broom potatoes appear to have no period of dormancy.

Senescence is delayed in potato vines affected with witches' broom.

In 42 per cent of the 142 trials made, potato witches' broom was transmitted by tuber-core grafts to 11 varieties of potato. It was transmitted twice by stem grafts.

Tuber core grafts with normal cores and seed pieces did not cause abnormalities in the resulting plants.

Seventy-one leaf-mutilation inoculations all failed to transmit potato witches' broom.

The disease was not transmitted by aphids or mealy bugs, nor was it transmitted by soil, or by root or leaf contact.

The appearance of the symptoms of viroses was often hastened by cutting off the ends of the stems of plants before signs of senescence appeared.

Witches' broom was transmitted by inarch grafts from potatoes to 17 tomatoes, from tomatoes to five other tomatoes, and from tomatoes back to two potatoes.

The main symptoms of tomato witches' broom are: Flavescence of the tops, marginal flavescence of the leaflets, extreme dwarfing, curling, flavescence and purpling of the leaflets, partial or total absence of leaf blades, spindling, flavescent axillary branches, and insipid little tomatoes.

Tomato witches' broom appears to be a new disease.

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Sections of tomato leaves showed a prominent morphological basis for marginal flavescence. The palisade cells were abnormally short, little chlorophyll was present, and the margins of the leaves were abnormally thin.

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THE COMPARATIVE NUTRITIVE VALUE OF THE PROTEINS OF LINSEED MEAL AND COTTONSEED MEAL FOR DIFFERENT ANIMALS [1]

By R. M. Bethke, G. Bohstedt, H. L. Sassaman, D. C. Kennard, and B. H. Edington, *Department of Animal Industry, Ohio Agricultural Experiment Station*

INTRODUCTION

There is need for information which will make possible the application of the knowledge of the qualitative differences in proteins to the selection of economical rations for farm animals. It is generally understood that animal protein is of higher quality than vegetable, and that a combination of the two is more efficient than vegetable protein alone. This knowledge is commonly made use of in pork and egg production. Within recent years, however, more information has been accumulating on the use of vegetable proteins, which tends to show that protein concentrates of vegetable origin can be frequently used to advantage to the partial or entire exclusion of the animal protein fraction. In this respect the two vegetable protein feeding stuffs, linseed meal and cottonseed meal, have held a great deal of interest and are extensively used as feeds for livestock.

In many feeding trials, especially those conducted in the North, linseed meal has been used as a standard protein feed, since it was felt that the protein of cottonseed meal was poorer in quality and that the cottonseed meal was of a toxic character. There is, therefore, no entire agreement as to the respective protein value of these two meals. In the majority of the experiments these vegetable protein feeds were investigated without taking into consideration feed consumption. In many instances the respective meals were taken from different shipments or sources and unknown factors were thus introduced, such as variable toxicity and nutritive value, which detracted from the clarity of the results. Hence, a further study of the comparative nutritive value of the proteins of cottonseed and linseed meal was considered necessary; such a study constitutes the subject of the present report.

REVIEW OF LITERATURE

The digestibility of the proteins of cottonseed meal is reported by Fraps (*4*) [2] to be 88.4 per cent in the case of sheep and steers, and by Lindsey and his coworkers (*8*) as 82.19 to 84 per cent for sheep. Mendel and Fine (*14*), who employed dogs as experimental animals, state that the protein of cottonseed flour is digested to the extent of 67 to 75 per cent, compared to 91 per cent for the proteins of meat. Rather (*23*), using men as subjects, found an average protein digestibility of 77.6 per cent for cottonseed meal and 78.4 per cent for cottonseed flour, in contrast to 96.6 per cent for the protein of meat.

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[2] Reference is made by number (italic) to "Literature cited," p. 870.

In comparing the nutritive value of the proteins in cottonseed meal, alfalfa hay, and corn, Nevens (*17*) found that when one of these feeds furnished the sole source of protein in an otherwise complete ration, to the extent of 10 per cent, the utilization of the proteins for the growth of albino rats was 66, 62, and 49 per cent, respectively. That cottonseed meal and flour were satisfactory sources of protein for the growth of albino rats, when these feeds furnished 18 per cent or more protein in the ration, was found by Richardson and Green (*24, 25, 26*). Osborne and Mendel (*19*) also reported that normal growth was procured for a considerable period in rats when 18 per cent of cottonseed globulin was fed in the ration. These latter investigators (*18*) found cottonseed flour of value in supplementing the proteins of corn gluten for growth of chickens. In other studies on the value of certain proteins and protein concentrates as supplements to corn gluten, these authors (*20*) demonstrated that the proteins extracted from cottonseed flour by a sodium hydroxide solution were efficient supplements to the proteins of corn gluten for the growth of rats. McCollum and Simmonds (*11*) reported that rats maintain their body weight when fed a ration containing 6 per cent of protein derived from cottonseed.

In studying the relation of the quality of proteins to milk production, Hart and Humphrey (*5*) found that the proteins of gluten feed, linseed meal, distillers' grains, and cottonseed meal were of equal value in supplementing the proteins of corn meal and alfalfa hay. Later in similar experiments (*6*) the proteins of distillers' grains, linseed meal, and gluten feed proved more efficient than cottonseed meal protein. The proteins of the feeding stuff tested formed but 40 per cent or less of the protein content of the ration, and the results were calculated upon the basis of total nitrogen absorbed by the animals.

Maynard, Fronda, and Chen (*13*), in studying the protein efficiency of combinations of corn meal and certain vegetable protein feeding stuffs, found that a mixture of corn meal and cottonseed meal was as efficiently utilized for growth of rats as a similar protein combination of corn meal and linseed meal.

That cottonseed and cottonseed meal may prove injurious to different species of animals has been shown by numerous investigators. The first assumption that the high protein content of cottonseed meal is responsible for its harmful effects was disproved by Dinwiddie (*3*), who showed that this theory was not supported by a study of the recorded feeding trials. Withers and Brewster (*30*) first attributed the toxic principle of cottonseed meal to a certain group of the protein molecule which contained loosely bound sulphur. Later work by Withers and Carruth (*31, 33*) led them to conclude that the toxicity was due to the presence of "gossypol." They were also of the opinion that gossypol may be changed to a nearly related substance, "D-gossypol," which possesses somewhat different chemical properties. The reduction of the toxicity of cottonseed meal by heat was believed by these investigators to be due to a change in the gossypol protein compound which renders it nontoxic or impossible for the animal to digest. These workers (*32*) also observed that iron salts have an antidotal action on cottonseed-meal poisoning in swine and rabbits. The theory that cottonseed meal injury is due to the pres-

ence of gossypol is strengthened by the work of Schwartze and Alsberg (*27*).

In a series of feeding experiments with rats, Osborne and Mendel (*19*) and Richardson and Green (*24, 25, 26*) observed no toxic effects with cottonseed meal or flour, but the cottonseed kernels proved injurious. Nevens (*17*) likewise found no toxic effects from feeding cottonseed meal to rats.

The feeding value of linseed meal for livestock has been demonstrated on numerous occasions. That this feed carries proteins which are of supplemental value to carbonaceous feeds is generally accepted by investigators and feeders. Henry and Morrison (*7, p. 723*) report an average digestibility of 89 per cent for the proteins of linseed meal. McCollum, Simmonds, and Parsons (*12*) observed that a ration of 25 per cent linseed meal, 1 NaCl, 1.3 $CaHPO_4$, 2 butterfat, and dextrin to 100 produced fair growth in rats. The growth and well-being of the rats was not comparable, however, to that on a ration containing 6 per cent protein from rye and 3 per cent from linseed meal. The latter ration also proved superior to 9 per cent rye protein. The combination of rye and linseed meal gave greater average growth than 8 per cent milk protein. These rations, in the light of present knowledge, were low in vitamins A and B. Nevertheless, they show the possible supplementing value of linseed meal. Further work by McCollum and Simmonds (*11*) shows that 8 per cent of flaxseed protein just serves to maintain body weight in the rat. This ration also was deficient in vitamin B.

That linseed meal may prove toxic to certain species of animals was reported by Almy and Robinson (*1*). These investigators found that ingested linseed meal brings about a nervous and excitable state in brook trout, causes them to become black and blind, and proves fatal to a large percentage of fish. They ascribed the injurious effect in large measure to hydrocyanic acid which is present in most linseed meals as the result of the hydrolysis of a cyanogenetic glucoside, phaseolunatin. The reason that most linseed meals apparently prove nontoxic is because decomposition of the glucoside does not take place in the stomach on account of the destruction of the enzyme by the high temperatures used in the expression of the oil.

EXPERIMENTAL METHODS

A large number of studies have been made with rats to ascertain the relative quality and adequacy of the proteins furnished by various sources. However, there is by no means entire agreement as to what constitutes a satisfactory method for determining the relative or comparative values of different protein sources, and many of the results reported by different investigators are conflicting. It was planned to attack the problem in several different ways to overcome the difficulties that might arise with each individual method, especially since the sources of protein did not differ greatly in quality, as they evidently do in many of the feeding stuffs of vegetable origin.

Protein sources have been compared, by many investigators, on the basis of their relative ability to cause growth or growth and reproduction without taking into consideration possible variations in food consumption. Osborne and Mendel (*21*) have shown that

any conclusions based on growth curves alone may be wholly misleading. McCollum (*9*), on the other hand, has pointed out that a rat eats according to its calorific requirements, and consequently food intakes should be comparable where rations are similar in calorific value. Under such conditions individual food intake records would add little of value. Other work has convinced the writers that other factors besides calorific requirements influence food intake, especially where rations differ markedly in physical character and ingredients. Questions of toxicity, palatability, and suitability of the ration to the animal arise. Under such conditions it is necessary to know whether an individual is actually eating enough food for growth before a given ration can be listed as unsatisfactory as to protein.

It was planned to pattern the first studies after those of Osborne and Mendel (*22*), feeding the rations ad libitum and then comparing results on the basis of gain per gram of protein eaten. Such a plan involves the feeding of rations adequate for normal growth with respect to all factors except protein. Also all results used for comparison must come from planes of protein intake producing fairly rapid growth, yet not average normal growth, since gain per gram is an accurate measure only in rations causing fairly good growth because it approaches zero as growth approaches zero. If, on the contrary, growth was comparable to normal there would be no way of ascertaining whether the protein intake was larger than necessary. Any excess protein would lower the true gain per gram. On this basis a plane of protein of approximately 10 per cent was decided upon. At this level rats of the writers' breeding will grow at about two-thirds to three-quarters of the normal rate when linseed meal and cottonseed meal are used as the sole source of protein.

The rats used were from two litters of six each which had been reared in the laboratory under standardized conditions. These were taken at the age of 25 days, weighing from 56 to 66 gm., and were equally distributed as to litter and sex. Each rat was confined in an individual cage and received the ration ad libitum.

EXPERIMENTS WITH RATS

Three rats from each litter were fed a ration of linseed meal 30 parts, cornstarch 59, salt mixture 4, Crisco 5, and cod-liver oil 2. The three remaining rats in each of the two litters received a similar ration in which the linseed meal was replaced by such a quantity of cottonseed meal as to make the total protein intake comparable. It was constituted as follows: Cottonseed meal 24.2, cornstatch 64.8, salt mixture 4, Crisco 5, and cod-liver oil 2. Vitamin B was supplied by 0.50 gm. of a brewer's yeast fed separately to each animal daily. The salt mixture employed was that described by McCollum (*10, p. 191*) with the addition of 0.25 per cent potassium iodide. Accurate accounts were maintained of the individual weekly food intakes.

The rats were continued on test for 15 weeks, because it seemed desirable that the experimental period should be as long as possible without extending into the period when the rate of growth starts to decrease. The results are shown in Table 1. The proteins of linseed and cottonseed meal appear to be equally efficient for growth of the rat when fed on a 10 per cent protein basis. No difference in the

behavior or appearance of the rats was observed. It is of interest to note that female rats utilized the protein of the rations less efficiently than the males. Although the yeast fed daily furnished some protein which supplemented those of linseed and cottonseed meal, the results, nevertheless, should be comparable, since the same quantity (0.50 gm.) was supplied each individual.

TABLE 1.—*Comparative efficiency for growth of rats of the protein in linseed meal and cottonseed meal*

Ration	Rat No.	Sex	Food intake	Protein intake	Gain in 15 weeks	Gain per gram of protein
			Gm.	*Gm.*	*Gm.*	*Gm.*
Linseed meal, 30 parts; starch, 59; Crisco, 5; salt mixture, 4; cod-liver oil, 2; 0.5 gm. yeast daily.	3036	Female	1,043	106.5	136.0	1.27
	3037	Male	1,111	113.5	180.0	1.58
	3038	Female	1,139	116.4	136.0	1.17
	3039	Male	1,091	111.5	171.0	1.53
	3040	Female	968	98.9	104.0	1.05
	3041	Male	1,307	133.5	179.0	1.34
Average			1,110	113.4	151.0	1.32
Cottonseed meal, 24.2 parts; starch, 64.8; Crisco, 5; salt mixture, 4; cod-liver oil, 2; 0.5 gm. yeast daily.	3042	Female	1,169	119.1	121.0	1.01
	3043	Male	1,187	121.0	193.0	1.59
	3044	Female	1,146	116.8	140.0	1.20
	3045	Male	1,148	117.0	156.0	1.33
	3046	Female	1,219	124.2	133.0	1.07
	3047	Male	1,182	120.5	162.0	1.34
Average			1,175	119.7	150.9	1.26

Before the foregoing study was completed the writers thought it advisable to obtain data on the digestibility of these two feed stuffs, having in mind that any variation in nutritive value might be partly or wholly due to the digestibility of the feed. Incidentally, the biological value of the feed stuffs was determined according to the method described by Mitchell (*15*). While the writers are aware of the shortcomings of any method which attempts to assign absolute figures to digestibility or biological values, they are, nevertheless, of the opinion that results secured in this way should prove of value in connection with other biological studies.

For these trials, one litter of 6 rats (3 males and 3 females) which had been reared under standardized laboratory procedure and weighed from 100 to 130 gm. were used. Each animal was confined in a 7-inch cylindrical wire metabolism cage provided with a false screen bottom, set in a crystallizing dish. The urine was absorbed by filter papers placed in the bottom of the dish. The feces were collected on a fine wire screen which was placed from one-half to three-quarters of an inch below the screen bottom. The urine and feces were collected daily and properly preserved to prevent loss of nitrogen. Food scattered by the animals was promptly removed from the filter paper and taken into account. The rations fed were of the same composition as those used in the first series, except that the yeast was fed mixed in the ration—6 per cent yeast replacing an equivalent quantity of starch. A total of two periods of seven days each was obtained with each animal on the two experimental rations. Periods 2 and 3 and 4 and 5 were run successively on the same ration. At the close of the third period, in each case, the order of the ration was reversed, and again two weeks' collection obtained. For example,

rat No. 1, which received the linseed-meal ration the second and third periods, was placed on the cottonseed-meal ration at the close of the third period. Likewise rat No. 4, which received the cottonseed-meal ration the second and third periods, was changed to the linseed-meal ration at the close of the third period. The low nitrogen periods at the beginning and end of the experiment were obtained with a synthetic ration containing the same amount of yeast (6 per cent) as was used in the experimental rations. A preliminary period of seven days preceded the collection periods whenever the rations were changed. The data are recorded in Table 2. The calculations were made according to the method of Mitchell, Beadles, and Keith (*16*), which takes into consideration the weight and food consumption of the animal in computing its metabolic and endogenous nitrogen. Considerable variation in digestibility and biological values occurred among the individual rats on the two rations. No significant difference was observed between the two meals when the average of the 12 trials in each case are considered. Apparently cottonseed meal and linseed meal, as used in this experiment, are equal in biological value and digestibility for rats.

TABLE 2.—*Nitrogen metabolism data for rats fed rations containing different percentages of nitrogen, together with data showing digestibility and biological value of linseed meal and cottonseed meal*

Ration	Rat No.	Initial weight	Final weight	Food intake	Food N	Fecal N	Urinary N	Digestibility*	Biological value
		Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*		
PERIOD 1									
Low nitrogen ration, containing 0.58 per cent N.	1	130	134	90.0	.....	0.160	0.275	.....	.....
	2	100	104	72.0	.....	.144	.285	.....	.....
	3	126	132	84.0	.....	.152	.291	.....	.....
	4	105	119	85.0	.....	.171	.264	.....	.....
	5	124	131	100.0	.....	.192	.241	.....	.....
	6	101	105	78.0	.....	.137	.292	.....	.....
PERIOD 2									
Linseed meal, containing 2.17 per cent N.	1	152	173	101.5	2.203	0.494	0.877	85.79	71.64
	2	111	124	64.5	1.400	.365	.611	83.07	75.92
	3	140	157	85.0	1.845	.349	.685	89.43	79.03
PERIOD 3									
Linseed meal, containing 2.17 per cent N.	1	173	189	109.0	2.365	0.503	0.933	86.93	73.30
	2	124	131	75.0	1.628	.352	.784	87.47	70.44
	3	157	178	101.0	2.191	.509	.839	85.12	75.66
PERIOD 4									
Linseed meal, containing 2.17 per cent N.	4	160	162	76.0	1.649	0.420	0.947	83.38	58.62
	5	212	220	92.0	1.996	.503	.985	84.27	65.46
	6	156	164	75.0	1.628	.362	.831	85.81	72.73

* Corrected for metabolic nitrogen.

TABLE 2.—*Nitrogen metabolism data for rats fed rations containing different percentages of nitrogen, together with data showing digestibility and biological value of linseed meal and cottonseed meal*—Continued

PERIOD 5

Ration	Rat No.	Initial weight	Final weight	Food intake	Food N	Fecal N	Urinary N	Digestibility	Biological value
		Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*		
Linseed meal, containing 2.17 per cent N.	4	162	172	82.0	1.779	0.484	0.904	81.51	64.62
	5	220	214	78.0	1.693	.356	.945	88.66	64.09
	6	164	170	75.0	1.628	.347	.773	86.73	78.47
Average								85.68	70.83

PERIOD 2

Cottonseed meal, containing 2.26 per cent N.	4	131	137	86.0	1.944	0.472	0.735	84.47	74.48
	5	147	180	121.0	2.735	.602	.839	86.65	77.55
	6	124	133	82.0	1.853	.402	.728	86.08	77.05

PERIOD 3

Cottonseed meal, containing 2.26 per cent N.	4	137	147	84.0	1.898	0.491	0.803	82.77	70.15
	5	180	204	124.0	2.802	.725	1.075	82.98	69.29
	6	133	144	88.0	1.989	.501	.822	82.55	73.75

PERIOD 4

Cottonseed meal, containing 2.26 per cent N.	1	214	232	119.0	2.689	0.675	1.066	82.74	73.53
	2	153	158	80.0	1.808	.482	1.128	81.97	53.98
	3	198	211	113.0	2.554	.657	1.049	82.30	72.74

PERIOD 5

Cottonseed meal, containing 2.26 per cent N.	1	232	254	120.0	2.710	0.679	1.115	82.77	73.70
	2	158	172	85.0	1.921	.451	.900	85.11	74.43
	3	211	247	127.0	2.870	.735	1.110	82.40	75.81
Average								83.57	72.20

PERIOD 6

Low nitrogen ration, containing 0.58 per cent N.	1	234	235	111.0	.....	0.196	0.511	.....	.....
	2	160	164	99.0	.....	.190	.478	.....	.....
	3	214	210	98.0	.....	.177	.502	.....	.....
	4	161	146	72.0	.....	.134	.359	.....	.....
	5	190	175	44.0	.....	.094	.340	.....	.....
	6	160	160	80.0	.....	.139	.449	.....	.....

Because of the fact that no difference was observed between linseed and cottonseed meal in digestibility, biological value, or in the gain of rats per gram of protein intake, it was planned to study the comparative supplementing value of these feeds when fed in combination with corn. While there is a possibility that these protein concentrates might supplement one protein in one order and another protein in the opposite order, corn was selected as the protein source for which supplementing sources were to be sought because it is one of the most extensively and commonly used feeding materials, and also because it is generally conceded to contain proteins of poor quality.

The linseed and cottonseed meals were fed in three varying levels with yellow corn in an otherwise complete ration, the meals in each case furnishing 6.8, 8.5, and 10.2 per cent protein. The rations were constituted as follows:

Ingredients	Ration 1125	Ration 1126	Ration 1127	Ration 1128	Ration 1129	Ration 1130
Yellow corn	72.00	67.00	62.00	72.00	67.00	62.00
Linseed meal	20.00	25.00	30.00			
Cottonseed meal				16.17	20.20	24.20
Salt mixture	4.00	4.00	4.00	4.00	4.00	4.00
Cod-liver oil	2.00	2.00	2.00	2.00	2.00	2.00
Yeast	2.00	2.00	2.00	2.00	2.00	2.00
Cornstarch				3.83	4.80	5.80

The salt mixture used was the one reported by McCollum (*10, p. 191*) with 0.25 per cent potassium iodide added. Two per cent of yeast was added to make certain that vitamin B would not be a limiting factor.

Several litters of young stock rats weighing from 47 to 60 gm. were divided into 6 groups of 4 each, 2 males and 2 females, and fed the above rations ad libitum. The animals were confined in standard wire cages provided with false screen bottoms. Accurate group feed-consumption records were maintained as well as individual weekly weights. The results are, in part, shown in Figure 1.

From this figure it is apparent that the rats grew at a nearly normal rate. The growth was fairly comparable on all six rations. Likewise, there was no difference in the behavior or appearance of the various lots. The rats fed cottonseed meal were fully comparable to those fed linseed meal. More cases of reproduction and slightly greater growth occurred in the lots fed cottonseed meal; however, this difference is not regarded as significant.

During the 24 weeks the various groups were on experiment, the 6 females in the three lots fed linseed meal gave birth to 11 litters, or a total of 76 young, of which 85 per cent were weaned at 24 days at an average weight of 25.4 gm. The 6 females on the cottonseed meal-corn combination gave birth to 15 litters or 105 young, of which 73 per cent were successfully weaned at 24 days and these weighed on an average 29.3 gm. It is interesting to note that the reproductive history of these rats does not coincide with the common belief among cattle feeders that cottonseed meal markedly interferes with normal reproduction.

EXPERIMENTS WITH RATS AND PIGS

All of the studies thus far had been carried out on one species, the rat, and indicated that with this animal the proteins of linseed meal and cottonseed meal were uniformly well digested, similar in biological value, and equally efficient in supplementing the proteins of corn. The writers desired, however, to obtain information on the comparative response of different species of animals when fed identical rations containing the two feeding stuffs in question. Accordingly,

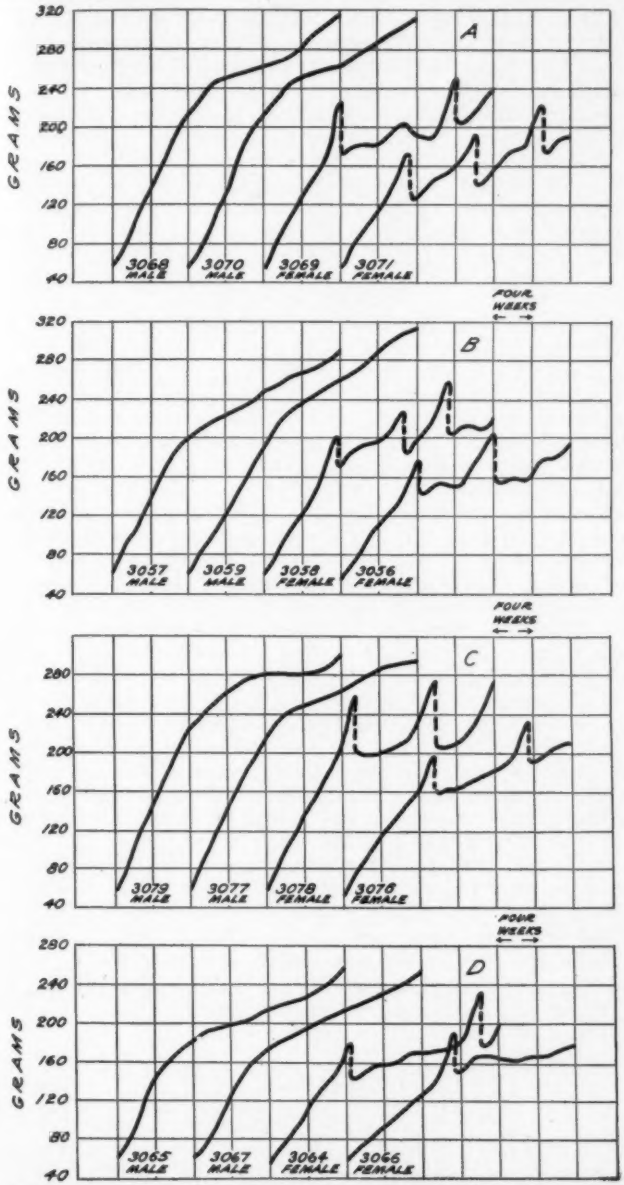

FIG. 1.—Graph showing the value of the protein of corn as a supplement to that of cottonseed meal and linseed meal in growth experiments with rats

A, ration 1128: Yellow corn, 72 parts; cottonseed meal, 16.17; salt mixture, 4; yeast, 2; cornstarch, 3.83; cod-liver oil, 2. B, ration 1125: Yellow corn, 72 parts; linseed meal, 20; salt mixture, 4; yeast, 2; cod-liver oil, 2. C, ration 1130: Yellow corn, 62 parts; cottonseed meal, 24.2; salt mixture, 4; yeast, 2; cornstarch, 5.8; cod-liver oil, 2. D, ration 1127: Yellow corn, 62 parts; linseed meal, 30; salt mixture, 4; yeast, 2; cod-liver oil, 2.

3 lots of 4 each of young stock rats, and 3 lots of 5 each of young weanling pigs weighing on an average 31.7 pounds, were fed identical rations. The pigs were confined indoors in concrete-paved pens and self-fed the respective ration. The rats were kept in the rat room in wire cages provided with false screen bottoms and fed the rations ad libitum. The rats were weighed every week and the pigs every two weeks. Fresh water was supplied daily in both cases.

One lot of rats and pigs received a ration of yellow corn 73 parts, linseed meal 25, and minerals 2.[3] A second lot received 77.8 parts yellow corn, 20.2 cottonseed meal (equivalent in protein content to 25 parts linseed meal), and 2 minerals. A third lot received a ration similar to that fed lot 1 except that the 25 parts of linseed meal was replaced by an equal quantity of cottonseed meal. The corn, linseed meal, and cottonseed meal were taken from the same source of supply as that in the previous rat studies.

The three lots of rats were continued on their respective rations for 24 weeks. The results are recorded in Figure 2. There was no discernible difference between the lot fed linseed meal and the lot fed cottonseed meal. The rate of growth was not comparable to that of rats fed corn-linseed meal or corn-cottonseed meal in rations fortified with cod-liver oil, yeast, and a synthetic salt mixture. Reproduction occurred among the lots on all three rations, with a high mortality of the young. Only in a few instances did the mother successfully nurse her young to 24 days. No difference was observed in this respect between the cottonseed-meal and linseed-meal lots.

The three lots of pigs, on the other hand, responded wholly differently. (Table 3.) From the beginning of the experiment the lot fed linseed meal ate more feed and continued to do better than the lots fed cottonseed meal. One pig in lot 2 (20.2 per cent cottonseed meal) died on the one-hundred and twentieth day. Another one toward the end of the experiment was inclined to go into convulsions when disturbed. In lot 3 (25 per cent cottonseed meal) two pigs died on the sixty-seventh and seventy-eighth days, respectively. On post-mortem examination all three animals showed a marked hemorrhagic condition of the mesentery—a characteristic of cottonseed-meal injury. The remaining animals were killed, after 132 days on experiment, for post-slaughter examination. No deviations from normal were observed at the time. However, the following morning it was noted that all the cottonseed-meal-fed pigs presented a distinctly yellowish coloration of the skin and internal fat. The linseed-meal-fed pigs, on the other hand, were normal.

TABLE 3.—*Data showing the relative value of cottonseed meal and linseed meal in rations fed to three lots of pigs of five each kept in dry lot from August 26, 1926, to January 5, 1927 (132 days)*

Lot and ration	Average daily feed	Feed required for 100-pound gain	Average initial weight of pigs	Average final weight of pigs	Average daily gain	Number of pigs that died
	Pounds	*Pounds*	*Pounds*	*Pounds*	*Pounds*	
Lot 1: Yellow corn, 73 parts; linseed meal, 25; minerals, 2	3.06	374.0	31.70	139.70	0.82	0
Lot 2: Yellow corn, 77.8 parts; cottonseed meal, 20.2; minerals, 2	2.32	504	32.00	105.80	.46	1
Lot 3: Yellow corn, 73 parts; cottonseed meal, 25; minerals, 2	2.26	439	31.50	107.20	.52	2

Special steamed bone meal 40, ground limestone 40, salt 20.

The difference in the response of the rats and pigs fed identical rations is of interest in that it shows that animals of different species may not respond alike to the same feeds. This does not necessarily imply that a difference in protein requirements or utilization exists between the two species. Questions of differences in palatability,

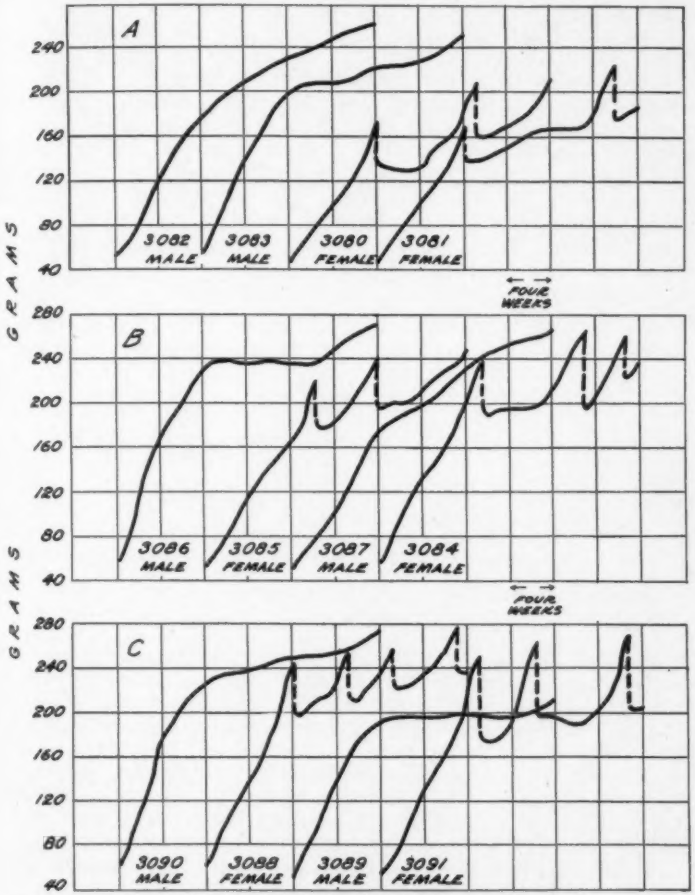

FIG. 2.—Graph showing the relative value of the protein of linseed meal and cottonseed meal in growth experiments with rats

A, ration 1131; Yellow corn, 73 parts; linseed meal, 25; minerals, 2.
B, ration 1132; Yellow corn, 77.8 parts; cottonseed meal, 20.2; minerals, 2.
C, ration 1133: Yellow corn, 73 parts; cottonseed meal, 25; minerals, 2.

suitability, and toxicity of the ration to the animal arise. The writers are of the opinion that the poor results obtained with cottonseed meal in the case of pigs were primarily due to toxicity and were not complicated by inferior protein. This belief is substantiated by other work at this station which has shown that cottonseed meal

can be profitably fed to pigs when combined with an animal protein feed such as tankage. These latter trials also indicate that the protein in cottonseed meal is equal in biological value to that in linseed meal.

EXPERIMENTS WITH CHICKS

Both cottonseed meal and linseed meal have been fed to laying hens on a few occasions with conflicting and inconclusive results. No experiments, however, have come to the writers' attention which attempted to compare the proteins of these two feeding materials in the case of the young growing chick. The latter represents a rapidly growing and rather delicate species which responds very readily to poor or good nutrition. Accordingly, it was planned to test the comparative protein efficiency of linseed meal and cottonseed meal when fed to chicks alone and when fed in combination with an animal protein in an otherwise complete ration.

One-week-old White Leghorn chicks of the station stock were divided into four lots of 25 each and placed in 3 by 6 foot experimental pens located indoors. Pine shavings, renewed weekly, were used as litter. The rations were compounded as follows:

	Lot 1	Lot 2	Lot 3	Lot 4
Yellow corn	43. 5	49. 3	50. 3	53. 2
Wheat middlings	20. 0	20. 0	20. 0	20. 0
Linseed meal	29. 5		14. 7	
Cottonseed meal		23. 7		11. 8
Meat scraps (50 per cent protein)			10. 0	10. 0
Bone ash	4. 0	4. 0	2. 0	2. 0
$CaCO_3$	1. 0	1. 0	1. 0	1. 0
NaCl	1. 0	1. 0	1. 0	1. 0
Cod-liver oil	1. 0	1. 0	1. 0	1. 0

These were fed in the form of a dry, finely ground mash, with water to drink. The rations of lots 1 and 2 represented 10 per cent protein in the form of linseed meal and cottonseed meal, respectively. In the case of lots 3 and 4 one-half or 5 per cent of vegetable protein was replaced by an equal quantity of animal protein. The bone ash in the rations of lots 3 and 4 was reduced to compensate for the bone contained in the meat scraps, since it was desired to maintain approximately the same calcium intake in all four groups.

The results of the chick experiment are summarized in Table 4. At a 10 per cent protein level cottonseed meal proved greatly superior to linseed meal for promoting growth and livability of chicks. Replacing one-half of the two vegetable proteins with an animal protein such as meat scraps (lots 3 and 4) made for a marked improvement in growth and livability. The combination of meat scraps and cottonseed meal proved to be better in this respect than that of meat scraps and linseed meal. Cottonseed meal alone (lot 2), however, was equal to the meat scraps-linseed meal mixture (lot 3). The chicks in lot 1 (10 per cent linseed-meal protein) exhibited a marked intestinal disorder which undoubtedly proved to be a contributing factor to the high mortality and slow growth.

This marked gastrointestinal disturbance was not observed in lot 3, where 5 per cent of the linseed-meal protein was replaced by an equivalent quantity of animal protein. However, the equivalent protein in form of cottonseed meal and meat scraps proved better than the linseed meal-meat scraps combination. Post-mortem examination of the chicks that died revealed nothing of a toxic nature which could be attributed to the meals. On these bases and under the conditions of the experiment it would appear that the protein in cottonseed meal was superior to that in linseed meal for growth of young chicks.

Further work by Kennard [4] at this station indicates that linseed meal may serve as a fairly efficient source of protein for egg production—proving equal to meat scraps in one trial. This fact suggested that probably the age or maturity of the bird was a factor in the difference in response between the chick and hen. To test this hypothesis the writers placed on experiment two lots, 10 each, of 12-week-old White Leghorn pullets. These had been reared indoors on a standard chick ration and were normal in all respects. They were fed rations similar in composition to lots 1 and 2 of the foregoing experiment. Pine shavings, renewed weekly, were used as litter. Water was supplied as a drink.

TABLE 4.—*Data for 4 lots of chicks of 25 each kept for 11 weeks on rations containing different quantities of linseed meal or cottonseed meal protein, fed without and with the addition of animal protein*

Age of chicks (weeks)	Lot 1, fed 10 per cent linseed-meal protein		Lot 2, fed 10 per cent cottonseed-meal protein		Lot 3, fed 5 per cent linseed-meal protein and 5 per cent meat-scraps protein		Lot 4, fed 5 per cent cottonseed-meal protein and 5 per cent meat-scraps protein	
	Number surviving	Average weight	Number surviving	Average weight	Number surviving	Average weight	Number surviving	Average weight
		Gm.		*Gm.*		*Gm.*		*Gm.*
1	25	69.9	25	70.2	25	69.2	25	70.0
3	23	97.0	25	121.0	22	115.2	23	148.0
5	17	100.7	25	191.5	20	171.7	22	247.7
7	10	139.0	*23	312.5	*20	291.0	*22	424.7
9	10	167.5	20	449.0	20	422.5	21	567.8
11	9	227.2	20	574.5	19	561.0	21	779.9
12	8	271.8	20	648.3	18	613.1	21	862.3

* Cockerel and pullet weights averaged separately after seventh week.

The birds were continued under this management for nine weeks. One bird in each lot died from bronchitis. The gain per bird during the course of the experiment was 311 gm. for those fed linseed meal and 374 gm. for those fed cottonseed meal. Although little significance is attached to the difference of 63 gm. in favor of the birds fed cottonseed meal, it is interesting to note that those fed linseed meal made comparatively small gains the first six weeks. The gains of those receiving cottonseed meal, on the other hand, were gradual. The total feed consumption of the two lots was practically equal. This suggests that maturity of the bird may be a factor in determining the relative nutritive value of these two protein feeds. This fact requires further experimentation.

[4] Unpublished data.

EXPERIMENTS WITH CALVES

In addition to the data secured with rats, pigs, and chicks, an experiment involving the feeding of these two protein concentrates to calves was carried out. Fourteen beef calves of the Aberdeen-Angus breed, averaging 342 pounds in weight, were fed in 2 lots of 7 each for a period of 280 days. The lots were comparable in weight, age, and sex. Obviously the feed mixtures as a whole could not be identical with those of the rats, pigs, or chicks. Alfalfa hay and corn silage were added. The proteins of these roughages in addition to those of corn were of necessity complicating factors. No concise conclusions as to the biological values of the proteins of cottonseed meal and linseed meal could be drawn when fed in combination with hay, silage, and corn, because the protein intake in the form of the two meals represented but a small portion of the total, and consequently their respective values were masked to a much greater extent than when fed alone or in combination with corn. Nevertheless, the data with calves are presented to show what results might be expected with the same feeds, particularly cottonseed meal, that from all indications had variable effects on different species of animals.

The 2 lots of calves received a ration of alfalfa hay, corn silage, and a grain mixture of 9 parts yellow corn and 1 part of either of the protein feeds. The corn and the cottonseed and linseed meals were from the same shipment as those used in the foregoing experiments with rats, pigs, and chicks. Both the roughages and grain mixture were fed ad libitum.

The average daily feed consumption of lot 1 consisted of 6.72 pounds ground corn, 0.71 pound linseed meal, 4.12 pounds alfalfa hay, and 4.86 pounds corn silage, possessing a nutritive ratio of 1:6.6 The individuals in lot 2 consumed on a daily average 6.04 pounds ground corn, 0.64 pound cottonseed meal, 3.71 pounds alfalfa hay, and 4.41 pounds corn silage having a nutritive ratio of 1:6.3.

On these rations, fed for 280 days, the calves of lot 1 (linseed meal) made an average daily gain of 1.70 pounds, and those of lot 2 (cottonseed meal) 1.66 pounds. The feed required per unit gain was slightly less for those fed cottonseed meal (lot 2). No unfavorable effects that might be traced to the rations were noticed in either lot. In numerous experiments with fattening beef cattle at the Wisconsin,[5] Pennsylvania (*29*), Nebraska (*28*), Kansas,[5] and Iowa (*2*) stations, linseed meal, with few exceptions, has produced greater and more economical gains than has cottonseed meal. The results of the writers' one trial reported above indicate, however, that cottonseed meal and linseed meal do not greatly differ in feeding value when fed to beef calves in combination with alfalfa hay, corn silage, and corn.

DISCUSSION

When the data are considered in toto it is evident that not all species of animals respond alike when fed either linseed meal or cottonseed meal. Results with rats indicate that the proteins of these two feed stuffs are equally well digested, possess the same

[5] Unpublished data.

biological value, and are equally efficient in supplementing the proteins of corn, when fed on the same protein basis. Obviously, in the case of this species the two feeds would be of equal nutritive value. A review of the literature, on the other hand, reveals many discrepancies and conflicting results as to the relative nutritive value of linseed meal and cottonseed meal in livestock husbandry. If the variable results are due to an actual difference in the protein value of these feeds apparently similar differences should have been found in the case of the rat. Since no differences were noted, it is assumed that the discrepancy in the reported results may be explained, wholly or in part, on the basis of toxicity or species variation. This assumption is strengthened by the fact that the pigs and chicks in the writers' trials responded differently from rats or calves fed the same meals. In the case of the pigs the poor results with cottonseed meal can be explained upon the basis of toxicity. This explanation, however, will not account for the difference in response of the rats and chicks. With the last-named species the data indicate that the protein of cottonseed meal is superior to that of linseed meal for growth. Other unpublished data show that linseed meal may serve as a fairly efficient source of protein for egg production. This fact suggests that animals within the same species may react differently, depending upon age or a particular function. Obviously, not all species of animals are affected alike by the same food, and the question of how far the results of nutrition studies obtained with one species of animal are applicable to other species becomes a serious one. Investigation has shown that the vitamin requirements of different species vary. Likewise, the question of energy and mineral requirements between different species or a particular function must be given consideration. With some exceptions, a protein which proves of high biological value in case of the experimental rat is commonly considered of equal value for farm stock. While there is no evidence which would definitely show that animals differ in their amino acid requirements, the writers' data indicate that a difference in response to protein feeding may be observed. Whether this difference is one of protein digestion, utilization, or amino acid content remains to be determined. The final decision as to the quality or value of any feed must rest upon experiments with the species in question and with respect to a particular function such as growth, milk production, or egg production. Accordingly, feeders as well as investigators must exercise caution in attempting to interpret the nutrition of one species in terms of another.

SUMMARY

The proteins of linseed meal and cottonseed meal were equally well digested, possessed the same biological value, and were equally efficient in supplementing the proteins of corn, when fed on the same protein basis to young rats.

Rats and pigs did not respond alike when fed identical rations containing linseed meal and cottonseed meal. The latter proved toxic to pigs.

The feeding of linseed meal and cottonseed meal to growing chicks, either as the chief source of protein or in combination with an animal protein (meat scraps), showed that the proteins of cottonseed meal

were more efficient than those of linseed meal. No toxic effects were observed with this species.

Evidence is presented which indicates that the age or maturity of the chicken may influence its response to linseed-meal or cottonseed-meal feeding.

Cottonseed meal and linseed meal were of nearly equal value when fed to beef calves in combination with alfalfa hay, corn silage, and corn.

It is pointed out that not all species of animals respond alike to linseed-meal or cottonseed-meal feeding, and that the conflicting results obtained with these two feedings stuffs may, wholly or in part, be accounted for on the basis of toxicity or species variation.

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CORRELATED INHERITANCE IN KANRED × SEVIER VARIETIES OF WHEAT[1]

By GEORGE STEWART

Agronomist in Charge of Plant Breeding, Utah Agricultural Experiment Station

INTRODUCTION

In a previous study[2] the writer made a critical analysis of segregates from the wheat cross Sevier × Federation. The data indicated a single major factor and a series of minor modifying factors for the inheritance of head density. Both the parents used in this cross were of intermediate head density, but some of the F_3 progenies had heads much more dense, and others had heads much more lax, than those of either parent. Segregates with the head density of Federation, the less dense of the two parents, were recovered in a few cases, but out of 321 F_3 progenies studied only 1 had a head density that even approached that of Sevier, and it was far from certain statistically that the density of Sevier was recovered in this segregate. It is more accurate to say that this was the only one of the 321 F_3 families studied which was not definitely different statistically from the Sevier parent in head density.

Awn inheritance also showed a behavior not hitherto observed. Federation was awnless in the usual sense, while Sevier was fully awned. Some homozygous F_3 families were obtained similar to one parent and some similar to the other parent; two intermediate forms of partly awned homozygous segregates were also obtained but in regularly smaller numbers than in the parental types. This and the ratios in the segregating types led to the suggestion of two factors for awns so linked as to produce gametes[3] in the ratio 1.8:1:1:1.8. Good fits by the X^2 method were obtained in two families and not a bad fit in a third family.

Simple correlation coefficients (r) and correlation ratios (η) were calculated for a number of characters. It was concluded that:

> In view of the consistently significant correlations, as judged by their probable errors, between the two stable and definitely inherited characters of spike density and awn classes, it seems reasonable to conclude that there is a strong suggestion of linkage between the factors for these two characters.

The cross Kanred × Sevier, made previously for the purpose of securing better winter wheats, fortunately involved just the right characters for a careful testing of the suggested linkage between the factors for awns and those for spike density.

The following study is therefore a by-product of an economic plant-breeding project. Not many data were taken in F_2, but since every F_2 plant was represented by an F_3 progeny no trouble was

[1] Received for publication Mar. 12, 1928; issued July, 1928. Contribution from the Department of Agronomy, Utah Agricultural Experiment Station. Approved for publication by the director of the station.

[2] STEWART, G. CORRELATED INHERITANCE IN WHEAT. Jour. Agr. Research 33: 1163–1192, illus. 1926.

[3] STEWART, G. INHERITANCE OF AWNS IN CROSSES INVOLVING SEVIER AND FEDERATION WHEATS. Jour. Amer. Soc. Agron. 20: 160–170. 1928.

encountered in making the genetic studies required to test the correlation previously suggested. The number of parent rows was, however, fewer than would have been the case had the test been definitely planned for correlation studies. Nevertheless, there were enough of these for reasonable comparison.

EXPERIMENTAL DATA

SCOPE OF THE INVESTIGATION

The investigational work here reported has to do with—

1. Inheritance studies of (1) spike density, (2) awn length, (3) plant height, (4) number of spikelets, (5) number of culms, (6) grain color, and (7) glume color.
2. Correlation between head density and awn length.
3. Simple, partial, and multiple correlations between the following characters: (1) Spike density, (2) awn length, (3) plant height, (4) number of spikelets, and (5) number of culms.

DESCRIPTION OF PARENTS

The parents used were Kanred and Sevier (pure line No. 59), a variety recently discovered[4] in Sevier County, Utah. So far as the writer is aware, this is the only region where Sevier wheat is grown commercially, though it has been recently introduced into the upper Snake River Valley of Idaho.

Sevier

The variety Sevier has commercial importance in the Sevier River Valley of Utah, where occasionally there is some black stem rust. The straw is extremely weak, and the grain lodges badly, although unless lodging is extremely severe there is little loss, for Sevier seems to have a certain amount of resistance to the physiologic forms of *Puccinia graminis tritici* that occur in this region. It is extremely high yielding under favorable conditions, is somewhat drought resistant, and is also thought to be slightly resistant to alkali, though this has not been proved. The spike is awned and is somewhat laterally compressed, and though considerably more dense of spike than is Kanred, Sevier can not be classed as a club wheat. The glumes are bronze but not dark bronze. The kernels are white, and in some pure lines are almost as hard as those of durum wheats, whereas in others the kernels are soft. In the pure line used as a parent of the hybrids studied the kernels are hard but not so hard as in some of the other lines. The grain is held firmly in the chaff and no amount of weathering seems to cause shattering, a fact which makes it a desirable parent to use in spring-wheat crosses both with Federation and Dicklow, as these two varieties both shatter considerably, and with Turkey or Kanred, which shatter slightly if left standing long after cutting readiness.

Kanred

The Kanred variety resulted from a pure-line selection from Crimean wheat at the Kansas station. It resembles ordinary Turkey rather closely, but differs from it visibly in having appreciably longer beaks on the glumes. Kanred is very important in the central Great Plains and has recently increased rapidly in the dry-farming sections

[4] Stewart, G. Sevier wheat. Jour. Amer. Soc. Agron. 15: 385-392. 1923.

of the central Rocky Mountain region. It is known to be immune to 11 of the common physiologic races of black-stem rust and to be more drought resistant in the central Great Plains than is common Turkey. In parts of Utah it has given higher acre yields than Turkey, otherwise the best commercial variety for the region, and is thought to be somewhat more winter hardy. It has the winter growth habit and is fully awned. It has a lax to mid-dense fusiform spike and a hard dark red kernel. The straw is considerably shorter than that of Sevier, but is nearly, though not quite so much, inclined to lodge. The chaff is white with faint bronze markings.

GENETIC STUDY

The very promising yield obtained from various segregates of crosses in which Sevier was one of the parents, and the frequent occurrence of recombinations of genetic factors[5] whereby segregates were obtained which were more resistant to *Puccinia graminis tritici* than either parent, led to an attempt to secure valuable wheat strains from the cross of Sevier on Kanred, which is probably the best commercial winter wheat of the region.

The cross was made at Logan, Utah, during the summer of 1923, between a pure line of Kanred and pure line No. 59 of Sevier. About 20 F_1 kernels were obtained and all were seeded that fall about 1 foot apart each way in a well-fertilized seed bed. The F_1 plants grew vigorously and yielded abundantly, producing from 800 to 2,500 kernels each, only a part of which were sown. Ten of the F_1 plants were harvested and grains from them seeded in the fall of 1924. The plants were spaced about 4 inches in rows 1 foot apart. The F_2 plants were studied from the standpoint of straw strength. Just before harvest two families which seemed to stand up better were selected for further study. The two F_2 families selected bore the pedigree numbers 15a–4 and 15a–7. The plants of each family were classified roughly as to head density and color of chaff, and accurately as to grain color. In the fall of 1925 an F_3-progeny row was seeded with the grain of each F_2 plant. There were seeded about 40 kernels in each F_3-progeny row. The rows were 1 foot apart, and the kernels were spaced 3 to 4 inches apart in the row. The data were taken on the F_3-progeny rows at harvest in July and August, 1926.

Spike Density

The F_1 plants had spikes that were of intermediate density, somewhat like those of the Sevier parent. No measurements, however, were taken in this case either of the F_1 or of the F_2 plants. In F_2 there was a notable transgressive segregation, some plants having considerably more dense spikes than either parent. The extremely lax spikes were not far different from the most lax spikes of the more lax parent, Kanred. In F_3 the density of each plant in each progeny was measured, and the mean of the 30 or 40 plants in each progeny used as the true classification of the F_2 plant from which it was descended.

The spike density was obtained by measuring carefully on a leading spike of each plant the length of 10 internodes along the central part of the rachis. The rachis internodes at the extreme base of the spike

[5] Stewart, G. Origin of a segregate resistant to black-stem rust in a cross between two susceptible plants. Amer. Nat. 62: 188–191. 1928.

and those at the extreme apex of the spike were avoided for the reason that they often vary both considerably and irregularly from those more centrally located. Measurement began, therefore, with the third or fourth spikelet internode from the bottom of the spike. It was found most convenient to take the measurement from the base of the third or fourth spikelet internode to the base of the sixth internode above on the same side of the spike. This gave the length of 10 internodes. When the mean length of 10 internodes for an F_3 progeny row was calculated, the mean length of each spikelet internode if desired was secured merely by pointing off one decimal place.

Not only was the mean density of each progeny obtained, but the coefficient of variability for each individual progeny was calculated. In Table 1, the spike densities of the parental rows of Sevier 59 and of Kanred and of the F_3 progenies are given. The F_3 progenies are arranged into three groups: (1) Those homozygous for dense spikes, (2) those heterozygous for spike density, and (3) those homozygous for lax spikes. Spike density classes in millimeters to the spikelet internode are shown at the top of the table. On the extreme right the coefficients of variability classes (C. V.) are designated.

TABLE 1.—*Spike-density classes of the means of Sevier and Kanred parental rows and of the means of F_3 progenies, arranged according to coefficient of variability classes (C. V.) of the individual rows of Sevier and Kanred parents and of three groups of F_3 hybrid progenies: (1) those homozygous for dense spikes, (2) those heterozygous for spike density, and (3) those homozygous for lax spikes*

[Family 15a–7; grown in 1926 at Petersboro, Utah]

Strain	Number of plants in spike-density classes (millimeters)									Total	C. V. classes
	1.75	2.25	2.75	3.25	3.75	4.25	4.75	5.25	5.75		
Sevier 59				1						1	
			1	6						7	
Total			1	7						Mean.	8.46
Kanred								2		2	6
								5	1	6	9
											12
								2		2	15
Total								9	1	Mean.	9.46
Homozygous dense		4								4	6
	4	23								27	9
	1	11								12	12
		1								1	15
	1									1	18
Total	6	39								Mean.	9.83
Heterozygous					1					1	21
				3	1					4	24
			3	12	3	2				20	27
			4	15	8	1				28	30
			5	17	5					27	33
			1	6	3					10	36
			6	1						7	39
			1	1						2	42
			1		1					2	45
											48
				1						1	51
Total			21	56	22	3				Mean.	31.82
Homozygous lax							8	2		10	6
						5	9	8		22	9
							4	2	1	7	12
Total						5	21	12	1	Mean.	10.45

Table 1 proves that there were progenies which were homozygous for dense spikes and others homozygous for lax spikes, whereas the plants of intermediate spike densities were all heterozynous. The coefficients of variability were used to indicate homozygosity or heterozygosity. The mean coefficients of variability for Sevier 59 and for Kanred were 8.46 and 9.46 per cent, respectively. An F_3 progeny was regarded as homozygous when its coefficients of variability were not essentially greater than those of the parental rows. Sevier 59 had a range of 6.6 to 9.8 per cent. The range for Kanred was from 6 to 16.4 per cent.

In the homozygous dense F_3 progenies the range in coefficients of variability was from 6.5 to 18 per cent. Only two were higher than 13.5 per cent. The mean was 9.83 per cent. In the homozygous lax group the range was from 6 to 13 per cent with a mean of 10.45 per cent.

In the heterozygous progenies, the coefficients of variability are so high as to prove heterozygosity at a glance. The range is from 22 to 50 per cent with a mean of 31.82 per cent. While there was no extreme gap between the most variable progeny classed as homozygous dense (C. V. = 18 per cent) and the least variable progeny classed as heterozygous (C. V. = 22 per cent), there was evidence in the material itself that they did not belong in the same groups. At most only this one, the most variable, of the homozygous dense progenies could be questioned.

Examination of Table 1 also proves that there was transgressive segregation in the direction of greater spike density. There is not one of the homozygous dense progenies that is not more dense than the most dense row of the more dense parent, Sevier 59. The most dense row of Sevier had a mean spike density of 2.983 mm. to the spikelet internode, whereas the most lax row of the homozygous dense progenies was 2.488 mm. The number and distribution of the parent rows did not permit the measurement of the variability of the soil, but it is extremely doubtful that there was even one homozygous dense progeny that approximates Sevier in head density. Not only was the segregation in this respect transgressive, but it was so strikingly so that all homozygous dense progenies were appreciably more dense than the most dense row of Sevier.

The homozygous lax progenies were very similar in spike density to the rows of the more lax parent, Kanred. Some of the more compact progenies in this group were considerably more compact than the most compact row of Kanred. Transgressive segregation did not in this cross occur at the lax end of the segregation as it did in the previous cross between Sevier × Federation.

The corresponding data for family 15a–4 are given in Table 2. There is no real difference in any respect save that three F_3 progenies in the homozygous dense group were slightly less dense than any in family 15a–7. In the progenies breeding true for dense spikes the coefficients of variability ranged from 7.6 to 14.5 per cent, with a mean 10.91 per cent. Those homozygous for lax spikes had a mean coefficient of variability of 7.71 per cent, with a range of from 5.7 to 11.5 per cent. On the other hand, the coefficients of variability of the progenies heterozygous for spike density ranged from 20.9 to 43.2 per cent, with a mean of 31.1 per cent. As in family 15a–7, it was easy to distinguish the true-breeding progenies from the segregating ones by the size of the coefficient of variability.

TABLE 2.—*Spike-density classes of the means of Sevier and Kanred parental rows and of the means of F_3 progenies, arranged according to coefficient of variability classes (C. V.) of the individual rows of Sevier and Kanred parents, and of three groups of F_3 hybrid progenies: (1) Those homozygous for dense spikes, (2) those heterozygous for spike density, and (3) those homozygous for lax spikes*

[Family 15a-4; grown in 1926 at Petersboro, Utah]

Strain	Number of plants in spike density classes (millimeters)								Total	C. V. classes
	2.25	2.75	3.25	3.75	4.25	4.75	5.25	5.75		
Sevier 59			1						1	6
		1	6						7	9
Total									Mean.	8.46
Kanred							2		2	6
							5	1	6	9
									0	12
							2		2	15
Total							9	1	Mean.	9.46
Homozygous dense	1								1	6
	4	1							5	9
	11	1							12	12
	1	1							2	15
Total	17	3							Mean.	10.91
Heterozygous	1								1	21
			1						1	24
			3	3					6	27
		2	7	2					11	30
		3	4	2					9	33
		1	5						6	36
		1							1	39
			1						1	42
Total	1	7	21	7					Mean.	31.10
Homozygous lax						6	8		14	6
						2	2		4	9
						2		1	3	12
Total						10	10	1	Mean.	7.71

In family 15a-4, the least dense progeny had a mean spike density of 2.723 mm. to the spikelet internode. As compared with family 15a-7, this is considerably nearer the most dense row of Sevier, which had a mean density 2.983 mm. The next least dense F_3 progeny in this group was 2.511 mm., which is almost identical with the least dense one in family 15a-7. It can not be said, therefore, with any real assurance that in any true-breeding progeny in this cross was the density of the Sevier parent recovered. On the other hand, it could not be proved that this one least dense progeny with a mean density of 2.723 mm. was statistically less dense than the most dense row of Sevier—2.983 mm.

In family 15a-7 there are 187 F_3 progenies, of which 45 bred true for dense spikes, 39 bred true for lax spikes, and 102 segregated for spike density. This suggests a 1:2:1 segregation—that is, a difference of one factor. The calculation for closeness of fit on this hypothesis is given in Table 3.

TABLE 3.—*Closeness of fit of three groups of F_3 progenies on a 1:2:1 segregation*

[Family 15a–7; grown in 1926 at Petersboro, Utah]

Group	C	O	C−O	$(C-O)^2$	$\frac{(C-O)^2}{C}$
Homozygous dense	45	46.5	−1.5	2.25	0.0500
Heterozygous	102	93.0	9.0	81.00	.8710
Homozygous lax	39	46.5	−7.5	56.25	1.2097
			X^2=2.1291		P=0.3478

In family 15a–4 there were 77 F_3 progenies, of which 20 bred true for dense spikes, 21 for lax, and 36 segregated. The calculations for closeness of fit on a 1:2:1 segregation are given in Table 4.

TABLE 4.—*Closeness of fit of three groups of F_3 progenies on a 1:2:1 segregation*

[Family 15a–4; grown in 1926 at Petersboro, Utah]

Group	C	O	C−O	$(C-O)^2$	$\frac{(C-O)^2}{C}$
Homozygous dense	20	19.25	0.75	0.5625	0.0292
Heterozygous	36	39.50	2.50	6.2500	.1623
Homozygous lax	21	19.25	1.75	3.0625	.1591
			X^2=0.3506		P=very high

Since X^2 is less than 1, the probability is very high that the hypothesis is at least approximately correct. In family 15a–7, P= 0.3478—that is, in 35 cases out of 100 a worse fit might be expected due to chance alone. There also is a high probability that the 1:2:1 hypothesis fits the facts rather closely.

There seems, therefore, to be a genetic difference of one major factor for head density involved in this cross.

Table 5 proves that there is considerably more variability in spike density, as measured by the coefficient of variability both in the homozygous dense progenies and in the homozygous lax progenies, than in either parent. The coefficient of variability (C. V.) for the Sevier rows was 3.7 per cent and for the Kanred rows 2.83 per cent. In family 15a–7 the coefficients of variability for homozygous dense and for the homozygous lax progenies were 7.78 and 5.62 per cent, respectively. In family 15a–4 coefficients of variability for the homozygous dense and for the homozygous lax groups were 6.32 and 4.91 per cent. The same figures for the heterozygous groups in families 15a–7 and 15a–4 were 9.91 and 10.60 per cent, respectively.

TABLE 5.—*The range of mean spike densities and the mean of mean spike densities of Sevier and Kanred rows, and of three groups of F_3 progenies, together with the coefficients of variability (C. V.) of the means of Sevier and Kanred rows and of the F_3 progenies*

[Families 15a–7 and 15a–4: grown in 1926 at Petersboro, Utah]

Strain	Range of spike densities of parental rows and of F_3 progenies		Mean of spike densities of parental rows and F_3 progenies		C. V. of mean spike densities of parents and F_3 progenies	
	Family 15a–7	Family 15a–4	Family 15a–7	Family 15a–4	Family 15a–7	Family 15a–4
Sevier 59	2.983–3.375	2.983–3.375	3.199	3.199	3.70	3.70
Kanred	5.127–5.632	5.127–5.632	5.292	5.292	2.83	2.83
Homozygous dense	1.753–2.488	2.015–2.723	2.134	2.361	7.78	6.32
Heterozygous	2.615–4.121	2.852–4.045	3.265	3.250	9.91	10.60
Homozygous lax	4.445–5.533	4.696–5.688	4.854	5.080	5.62	4.91

Since the variability in the true-breeding progenies is about double that found in similar rows of parental material, it is apparent that there must be present, in addition to the single major factor for spike density, some minor factors which bring this about.

AWN LENGTH

Since both parents were fully awned, it is unlikely that any attention would have been given to this character had there not been the strong indication already noted of correlation between awn length and spike density. As it was, no data were taken in either F_1 or F_2. However, in F_2 general observations were made. It was thought that the compact heads bore somewhat regularly shorter awns that did the lax heads.

In the summer of 1926 the awns of each plant in each of the progenies were measured. The mean length of the 30 or 40 plants was used as the correct figure for the parental F_2 plant. The coefficient of variability for each individual progeny was then calculated. In order to ascertain whether the anticipated correlation between awn length and spike density really existed, correlation coefficients (r) and other correlation constants were calculated, with the results reported in a later section of this paper. Since a high correlation was found, the awn-length data given in Table 6 are classified into the three principal spike-density groups in which the progenies of given awn lengths were found. Even the gross grouping so made discloses the presence of a considerable correlation. The approximate centers of the progeny distribution for awn length in the groups which had dense spikes, which was heterozygous for spike density, and which had lax spikes, respectively, roughly arrange themselves in a diagonal line.

Table 6 also establishes clearly that Sevier 59 has much shorter awns than Kanred. The awns of Sevier are so appreciably shorter that there is a considerable gap between the Sevier row with the greatest mean length and the Kanred row with the least mean length. The F_3 progenies show a distinct segregation which yields progenies throughout almost the entire range between that row of Sevier with the shortest awn length to that row of Kanred with the greatest.

Segregation was not of such a nature that ratios between the various classes were obtained. With carefully grouped, replicated, and checked plantings it is likely that this could be done. However, the wide variation in awn length, as indicated by large standard deviations, would make this task one that called for refined statistical methods, both in the planting plan and in taking and calculating the data.

TABLE 6.—*Awn-length classes of the means of Sevier and Kanred parental rows and of the means of the F_2 progenies, arranged according to coefficient of variability classes (C. V.) of the individual rows of the Sevier and Kanred parents and of the F_2 hybrid progenies. In order to show the close correlation between spike density and awn length, the F_2 hybrid progenies are grouped according to the three spike-density groups: (1) Those homozygous for dense spikes, (2) those heterozygous for spike density, and (3) those homozygous for lax spikes*

[Family 15a–7; grown in 1926 at Petersboro, Utah]

Strain	Number of plants in awn-length classes (millimeters)																Total	C. V. classes
	51	54	57	60	63	66	69	72	75	78	81	84	87	90	93	96		
Sevier 59						1											1	6
					2													9
					3	1											3	12
				1	2												3	15
																		18
																		21
	1																1	24
Total	1			1	4	2											Mean.	13.48
Kanred												1			1	1	3	12
											1	1	3				5	15
											1						1	18
																		21
										1							1	24
Total										1	2	2	3		1	1	Mean.	15.65
Homozygous dense	1		2	3	7	3	1	4									8	9
				2	3	6	2	4	2								27	12
					1	1		1	1								8	15
					1												1	18
																	1	21
Total	1		2	5	12	10	3	9	3								Mean.	12.56
Heterozygous									1								1	9
							3	4	2	3	1			1			14	12
						9	8	8	11	7	3				1		47	15
				1	4	2	7	7	6	3	1						31	18
					1	2	4		1	1							9	21
																		24
								1									1	27
Total				1	5	13	22	20	21	14	5			1	1		Mean.	16.02
Homozygous lax								1	1	1	3	1		3			10	12
							1	3	5	3	3	3	2	1	1		22	15
							1			1	1	1	2		1		7	18
Total							2	4	6	5	7	5	4	4	2		Mean.	14.61

Similar data for family 15a–4 are given in Table 7. This table is as near an exact duplicate of Table 6 as could be hoped for. The only difference is that the awns of the F_2 progeny with the greatest mean awn length is 6 mm. shorter than the corresponding one in family 15a–7. (Table 6.) This is not surprising, however, in view of the fact that this family had in it less than half as many F_2 progenies, and also in view of the fact that it was grown on less productive land. Here the two Kanred rows with the greatest mean

awn length stand well out beyond the F_3 progeny having the greatest mean awn length. The defect of having too few parental rows prevents a definite conclusion that the F_3 progenies really lacked some of the awn length of the Kanred parent.

TABLE 7.—*Awn-length classes of the means of Sevier and Kanred parental rows and of the means of the F_3 progenies, arranged according to coefficient of variability classes (C. V.) of the individual rows of the Sevier and Kanred parents and of the F_3 hybrid progenies. In order to show the close correlation between spike density and awn length, the F_3 hybrid progenies are grouped according to the three spike-density groups: (1) Those homozygous for dense spikes, (2) those heterozygous for spike density, and (3) those homozygous for lax spikes*

[Family 15a–4; grown in 1926 at Petersboro, Utah]

Strain	Number of plants in awn-length classes (millimeters)																Total	C. V. classes
	51	54	57	60	63	66	69	72	75	78	81	84	87	90	93	96		
Sevier 59						1											1	6
																		9
					2	1											3	12
				1	2												3	15
																		18
																		21
	1																1	24
Total	1			1	4	2											Mean.	13.48
Kanred											1	1			1	1	3	12
											1	1	3				5	15
											1						1	18
																		21
										1							1	24
Total										1	2	2	3		1	1	Mean.	15.65
Homozygous dense			1	2	5	2	1	1									12	12
					1	2	1										4	15
			1		1												2	18
	1			1													2	21
Total	1		2	3	7	4	2	1									Mean.	14.16
Heterozygous							2			2							4	12
		1		2	5	5	2	4	2	2	1						24	15
					2	1		1	1								5	18
				1	1												2	21
					1												1	24
Total		1		3	9	6	4	5	3	4	1						Mean.	15.73
Homozygous lax											1						1	9
								2	3	2		4	2				13	12
										1	2		1				4	15
									1								1	18
						1											1	21
																		24
											1						1	27
Total						1		2	4	3	4	4	3				Mean.	14.02

HEIGHT OF PLANT

Height of plant was obtained by measuring each of the 30 or 40 plants in each parental row and in each F_3 progeny. The roots of the pulled plant were pressed firmly against a footboard nailed perpendicularly to a baseboard which was marked in centimeters. The height of the plant was read directly, to the base of the spike on the longest culm. It might have been more accurate, therefore, to have designated this character as the length of the longest culm. As with the other characters, the mean for the row was the figure used.

The shortest row of Kanred was 75.9 cm. tall and the tallest row 101.5 cm.; the mean height was 89.2 cm. The mean height of the shortest row of Sevier 59 was 94.9 cm., of the tallest row 113.1 cm., and the mean of all rows 102.6 cm. The height of the shortest F_3 progeny was 87.9 cm., the tallest one was 126.7 cm., and the mean of all was 107.1 cm. No F_3 progenies were obtained as short as the shortest Kanred row, but the tallest F_3 progeny exceeded the tallest Sevier row by almost as many centimeters as the shortest F_3 progeny exceeds the shortest Kanred row. There were too few parental rows to permit very accurate conclusions. While from casual examination of Table 8 it looks as if there were transgressive segregation in at least the tallest progeny, which was 13.6 cm. taller than the tallest row of Sevier, this difference is not much greater than the probable error.

TABLE 8.—*Frequency distribution of the rows of the Sevier and Kanred parents and of 187 F_3 hybrid progenies, arranged into classes according to mean height of the tallest culm and according to standard deviation classes (S. D.) of the individual rows of parents and of F_3 progenies*

[Family 15a-7; grown in 1926 at Petersboro, Utah]

Strain	Number of plants in plant-height classes (centimeters)																		Total	S. D. classes
	75	78	81	84	87	90	93	96	99	102	105	108	111	114	117	120	123	126		
Sevier 59								1	1	1		1	1	1					6	6
								1											1	9
								1											1	12
Total								3	1	1		1	1	1					Mean.	7.42
Kanred	1	1				1													3	6
		1	1					1	1										5	9
								1		1									2	12
Total	1	2	1			1	1	2	1	1									Mean.	8.67
F_3 progenies						4	5	12	12	14	18	15	11	1					92	6
					1	7	10	7	15	13	13	5	3	1	1				76	9
						1		3	2	2	1	1	1						11	12
									1							1			2	15
											1		1		1			1	4	18
												1							1	21
Total					1	12	15	22	30	29	33	22	16	2	2	1		1	Mean.	8.06

The distribution of the mean heights of the parental rows and of the F_3 progenies in classes, according to height and according to standard deviation (S. D.), is shown in Table 8. The frequency chart in this table shows a genetic difference in height between the two parents. The distribution of the mean heights of the F_3-progeny rows indicates a segregation for height. The nature of the segregation could not be accurately determined from the data secured.

NUMBER OF SPIKELETS

The number of spikelets was obtained by actual count, the sterile spikelets not being included. This set of data was taken from the same leading spike used for spike-density and for awn-length measurements. As in the other cases, the mean for the 30 or 40 plants in each parent row or in each progeny was the figure used.

The smallest number of spikelets to the spike on any row of Sevier 59 was 12.83; the highest number was 15.85; the mean was 14.78. For Kanred the lowest, highest, and mean numbers were 13.60, 17.25, and 14.45, respectively. In the F_3 progenies the lowest number of spikelets to the spike was 11.84 and the highest number 18.15. The mean was 15.77. Although the mean number of spikelets is higher in the F_3 progenies it is not significantly so, the difference being about one times the probable error.

There was, however, one F_3 progeny which had a smaller mean number of spikelets than any parental row and one which had more than any parental row. In Table 9, the distribution, according to spikelet-number classes arranged also in standard deviation classes (S. D.), brings out this apparent transgressive segregation. Two more classes are required at the higher end of the distribution for the F_3 progenies than for the highest parental row, which itself was three classes higher than the next highest parental row. The amount of variability does not differ materially in the two parents and in the F_3 progenies.

TABLE 9.—*Frequency distribution of the rows of the Sevier and Kanred parents and of 187 F_3 hybrid progenies, arranged into classes according to mean number of spikelets per leading spike of each plant and according to standard deviation classes (S. D.) of the individual rows of parents and of F_3 hybrid progenies*

[Family 15a-7; grown in 1926 at Petersboro, Utah]

Strain	Number of plants in spikelet classes												Total	S. D. classes
	12.75	13.25	13.75	14.25	14.75	15.25	15.75	16.25	16.75	17.25	17.75	18.25		
Sevier 59	1						1						1	1
			1	1	1	1	2						7	2
Total	1		1	1	1	1	3						Mean.	1.91
Kanred			1										1	1
			3	2		1				1			7	2
				1									1	3
Total			4	3		1				1			Mean.	1.99
F_3 families			1			2		4	2	1			10	1
			7	5	20	29	24	16	22	7	6		136	2
			1	4	4	4	9	8	4	4		1	39	3
														4
													1	5
Total			9	9	24	35	33	28	28	12	6		Mean.	2.12

There seems to be a segregation in the number of spikelets, though its nature could not be determined from the data available. The data indicate that very careful statistical work would be required to obtain the nature of the segregation.

NUMBER OF CULMS

The number of culms to the plant was obtained by actual count on all of the plants in each parental row and in each F_3 progeny. The mean for each row was used as representing properly the parent F_2 plant.

The mean number of culms per plant ranged from 8.97 to 12.12 in the rows of Sevier 59, and from 7.82 to 13.45 in the rows of Kanred.

The means were 10.12 and 10.43 for Sevier 59 and Kanred, respectively. In the F_3 progenies the range was from 7.40 to 14.89, and the mean was 10.56 culms per plant.

Table 10 shows the parental rows and the F_3 progenies grouped into classes for the mean number of culms and for the size of the standard deviations (S. D.). There seems to be a slight indication of transgressive segregation in the direction of a few rows with more culms to the plant. The mean standard deviation (S. D.), however, is no greater for the F_3 progenies than for the parents. It may be that the indication of transgressive segregation is only apparent, since the small number of parental rows probably does not show the entire range in their respective variabilities.

TABLE 10.—*Frequency distribution of the rows of the Sevier and Kanred parents and of 187 F_3 hybrid progenies, arranged into classes according to mean number of culms per plant and according to size of the standard deviation (S. D.) of the individual rows of parents and F_3 hybrid progenies*

[Family 15a-7; grown in 1926 at Petersboro, Utah]

Strain	Number of plants in culm number classes									Total	S. D. classes
	7	8	9	10	11	12	13	14	15		
Sevier 59			1							1	3
			2	1	1					4	4
			2			1				3	5
Total			5	1	1	1				Mean.	4.25
Kanred		1								1	3
		1	1		2					4	4
					1	1				2	5
	1			1			1			3	6
Total	1	2	1	1	3	1	1			Mean.	4.73
F_3 progenies				2						2	2
	2	11	12	9	8	1				43	3
	3	8	21	17	14	8	2	1		74	4
		2	10	11	13	8	3	2		49	5
				2	3	3		1		9	6
			1	1		1				3	7
											8
				1					1	2	9
				1						1	10
				2						2	11
					1					1	12
Total	5	21	44	46	39	21	5	4	1	Mean.	4.37

COLOR OF GRAIN

In 1926 each F_3 plant was classified as to grain color. The progenies were then grouped into true-breeding reds, true-breeding whites, and those segregating for red and white. The preliminary grouping indicated the likelihood of the three-factor differences for grain color. This led to a study of the nature of the segregation in each of the segregating rows. The calculated expectancy for each 64 plants on a three-factor basis is as follows:

True-breeding red grain	37
Segregating 63 red : 1 white	8
Segregating 15 red : 1 white	12
Segregating 3 red : 1 white	6
True-breeding white grain	1
	64

Examination of the data showed an excess of true breeding reds and a deficiency in the segregating classes. Since there were only 30 to 40 plants in each progeny, there would be several that should segregate 63 : 1, which would fail to show the one plant with white grain. In the fall of 1926, when F_4 progenies were seeded in order to get pure lines for rod-row testing, the rows were made long enough to include from about 100 to 110 plants. Altogether, 957 F_4 progenies were grown. Several of these came from each of 56 progenies which were true for red grain in 1926. This gave a good check on these 56 rows. Of these, 49 bred true for red grain; 6 segregated as 63 red : 1 white; and 1 segregated as 15 red : 1 white. In addition, two progenies which were intermediate between 63 : 1 and 15 : 1 proved to be segregating for 15 red : 1 white. The F_3 data on three other progenies left doubt as to whether they were segregating 15 : 1 or 3 : 1. Two of these proved to be 3 : 1 and one 15 : 1. Since only about half the F_3 progenies were tested, it would have been legitimate to double these corrections. This would have given an extremely close fit to the expected numbers in each class. However, in order to avoid all criticism, only the actually observed corrections were made. The results so obtained are given in Table 11.

TABLE 11.—*Number of F_3 progenies (1926) in each class based on 40 plants to the row, and the number of corrections obtained in F_4 (1927) on the basis of about 100 plants to the row*

[Family 15a-7; grown at Petersboro, Utah]

Class as to grain color	Number of F_3 progenies	F_4 corrections	Final count
True-breeding red	127		120
63 red : 1 white	* 12 (?2)	6	18
15 red : 1 white	24 (?2)	4	28
3 red : 1 white	* 15 (?3)	2	17
True-breeding white	3		3
	186		186

* Intermediate. Not sure as to classification.

Table 12 shows the closeness of fit for the color-of-grain data on the basis of a three-factor difference between the two parents.

TABLE 12.—*Closeness of fit of five groups as to grain color on a three-factor difference (37 : 8 : 12 : 6 : 1 ratio)*

[Family 15a-7; grown in 1926 and 1927 at Petersboro, Utah]

Group	C	O	C−O	$(C-O)^2$	$\frac{(C-O)^2}{C}$
Homozygous red grain	107.53	120	−12.47	155.5009	1.4461
Segregating 63 red : 1 white	23.25	18	5.25	27.5625	1.1855
Segregating 15 red : 1 white	34.88	28	6.88	47.3344	1.3571
Segregating 3 red : 1 white	17.44	17	.44	.1936	.0111
Homozygous white grain	2.91	3	−.09	.0081	.0028
			$X^2=4.0001$	$P=0.4060$	

It seems reasonable to conclude that the theory of a three-factor difference fits the facts, since a P of 0.41 reasonably approximates

the desired 0.50. If the corrections obtained on half the F_3 progenies were assumed to apply to the other half, P would then become 0.78. It is to be emphasized, however, that no such assumptions were made and yet the fit was good.

No F_4 selections were made from family 15a–4, on which account grain color segregations were not obtained.

Color of Glumes

Each plant in each F_3 progeny was classified for color of glumes. In family 15a–7 there were 38 families which bred true for bronze glumes and 50 which bred true for white glumes. The remaining 98 progenies segregated. This suggests a 1:2:1 ratio—that is, a one-factor difference. In family 15a–4 there were 16 true-breeding progenies for bronze glumes; 22 true for white; and 39 segregating for red and white. This also suggests a one-factor difference. The closeness of fits for this hypothesis are given in Tables 13 and 14.

Table 13.—*Closeness of fit of three groups of color of glumes compared to a 1:2:1 ratio*

[Family 15a–7; grown in 1926 at Petersboro, Utah]

Group	C	O	C−O	(C−O)²	$\frac{(C-O)^2}{C}$
Homozygous bronze	46.5	38	8.5	72.25	1.5538
Heterozygous	93.0	98	−5.0	25.00	.2688
Homozygous white	46.5	50	−3.5	12.25	.2634
			$X^2=2.0860$		$P=0.3554$

Table 14.—*Closeness of fit of three groups of color of glumes compared with a 1:2:1 ratio*

[Family 15a–4; grown in 1926 at Petersboro, Utah]

Group	C	O	C−O	(C−O)²	$\frac{(C-O)^2}{C}$
Homozygous bronze	19.25	16	3.25	10.5625	0.5487
Heterozygous	38.50	39	−.50	.2500	.0065
Homozygous white	19.25	22	−2.75	7.5625	.3929
			$X^2=0.9481$		$P=0.6189$

In family 15a–7, $P=0.36$, and family 15a–4, $P=0.62$. It is highly probable, therefore, that the segregation is in a 1:2:1 ratio—that is, determined by a single-factor difference.

CORRELATION STUDIES

Simple, partial, and multiple correlations were made of the mean values of F_3 progenies. As already mentioned, measurements or counts were taken on each F_3 plant in each progeny, and from these the mean of the entire progeny was calculated for the 187 F_3 progenies in family 15a–7 and for the 77 F_3 progenies in family 15a–4.

All possible correlations were made between the five characters on which observations were taken. The combinations were as follows: (1) Head density × awn length; (2) head density × height of plant; (3) head density × number of spikelets; (4) head density × number

of culms; (5) awn length × height of plant; (6) awn length × number of spikelets; (7) awn length × number of culms; (8) height of plant × number of spikelets; (9) height of plant × number of culms; and (10) number of spikelets × number of culms.

In family 15a–4, part of the F_3 progeny rows extended to a piece of shallow, stony, and less fertile land. The plants became noticeably shorter in a somewhat progressive but not very regular fashion in the region where the progeny rows with lax heads were growing. At first it was decided to abandon this family, but when the high correlation was obtained between spike density × awn length in family 15a–7, these data were taken on family 15a–4 also. There was reason for believing from previous work that the two characters of spike density and awn length were stable enough not to be widely influenced by somewhat poor soil. Accordingly, the observations were made and the data calculated as for family 15a–7. As will be seen from Table 15, the only coefficients of correlation that differ materially from those obtained in family 15a–7 are the ones involving height of plant, and only two of these could be considered erratic—(1) spike density × height of plant and (2) awn length × height of plant.

TABLE 15.—*Correlation coefficients (r), correlation ratios (η), their respective probable errors (P. E.), and Blakeman's test of linearity for various pairs of plant characters*

[Means of F_3 progeny rows, each of 35 to 40 plants, grown in 1926 at Petersboro, Utah]

Characters correlated	$r \pm$P. E.	$\frac{r}{\text{P. E.}}$	$\eta \pm$P. E.	$\frac{\eta}{\text{P. E.}}$	Blakeman's test
Spike density × awn length:					
15a–7	+0.6737±0.027	25.0	0.7006±0.025	28.0	1.9474
15a–4	+ .7619± .032	23.6	.8712± .026	33.5	1.9218
Spike density × height of plant:					
15a–7	+ .0696± .049	1.4	.3917± .042	9.4	3.908
15a–4	− .6001± .049	12.2	.7310± .036	20.4	2.7156
Spike density × number of spikelets:					
15a–7	− .3169± .044	7.1	.4676± .039	12.1	3.476
15a–4	− .2447± .072	3.4	.3406± .068	5.0	1.542
Spike density × number of culms:					
15a–7	+ .1238± .049	2.5	.3996± .041	9.7	3.852
15a–4	+ .0075± .077	.1	.3259± .069	4.7	2.1191
Awn length × height of plant:					
15a–7	+ .0985± .049	2.0	.2044± .047	4.3	1.8157
15a–4	− .4070± .064	6.3	.4980± .058	8.6	1.8661
Awn length × number of spikelets:					
15a–7	− .0629± .049	1.3	.2037± .047	4.3	1.9587
15a–4	− .016 ± .077	.2	.3185± .069	4.6	2.0692
Awn length × number of culms:					
15a–7	+ .1611± .048	3.4	.3586± .043	8.3	3.247
15a–4	+ .0377± .077	.5	.2982± .070	4.3	1.9239
Height of plant × number of spikelets:					
15a–7	+ .2273± .047	4.8	.4078± .041	9.9	3.422
15a–4	+ .4344± .063	7.0	.5024± .057	8.7	1.642
Height of plant × number of culms:					
15a–7	+ .3065± .045	6.9	.4401± .040	11.1	3.0599
15a–4	+ .1687± .075	2.3	.4671± .060	7.8	2.8333
Number of spikelets × number of culms:					
15a–7	+ .1950± .048	4.1	.2435± .047	5.2	1.474
15a–4	+ .2517± .072	3.5	.3195± .069	4.6	1.2796

The correlation coefficient (r) and the correlation ratio (η) were obtained for each of the 20 correlations. The respective probable errors and Blakeman's tests were calculated and tabulated as given in Table 15. Since spike density has been shown in previous papers and in this one to be a stably inherited character, its correlations with other characters are given first. Awn inheritance has been also shown to be stably inherited, and its correlations with the other characters are next in order. The correlations of other characters follow:

Spike density correlated with awn length gives in family 15a–7 a correlation coefficient (r) of $+0.6737 \pm 0.027$; in family 15a–4, $r = +0.7619 \pm 0.032$. In one case r is 25 times its probable error and in the other case 23.8 times. The correlation ratios (η) are 0.7006 ± 0.025 and 0.8712 ± 0.026 and the ratios of $\frac{\eta}{\text{P. E.}}$ are 28 and 33.5, respectively. Blakeman's tests for linearity in these two cases are 1.9474 and 1.9218, showing that the probability of the regressions being linear is about 1 in 4. These figures all denote clearly and definitely a high correlation between spike density and awn length, such as can be measured by r.

The nature of the correlation surfaces between spike density and awn length is shown in Tables 16 and 17. An examination of the frequency distributions in both tables will again emphasize the high degree of linear correlation and also the almost exact similarity between them.

TABLE 16.—*Correlation between the characters spike density and awn length*

[Family 15a–7; grown in 1926 at Petersboro, Utah]

Spike-density classes (millimeters)	Awn-length classes (millimeters)															Total
	51	54	57	60	63	66	69	72	75	78	81	84	87	90	93	
18				1	3											4
21	1		2	4	6	7	3	4	2							29
24					3	3		5	1							12
27					1	2	4	2								9
30					2	6	6	3	4	2				1		24
33				1	1	4	8	7	11	7	4					43
36					1	1	5	6	4	4	1	1			1	24
39								2	1	1						4
42								1					1			2
45							1		2		3			1		7
48								2	2	2	3	1	2	2	1	15
51							1	2	2	2	1	2	1			11
54												1		1	1	3
Total	1	0	2	6	17	23	28	34	29	18	12	5	4	5	3	187

$r = +0.674 \pm 0.027$.

TABLE 17.—*Correlation between the characters spike density and awn length*

[Family 15a–4; means of F_3 progenies grown in 1926 on rough, irregular, and relatively nonproductive soil, at Petersboro, Utah]

Spike-density classes (millimeters)	Awn-length classes (millimeters)													Total
	51	54	57	60	63	66	69	72	75	78	81	84	87	
21			1	1	1	1								4
24	1	1	1	2	6	3	2							16
27								1						1
30				3	5	1	2	1	1					13
33					3	3		1	2	3	1			13
36					1	1	2	2						6
39						1		1		1				3
42														0
45														0
48										1	1	2	3	8
51						1		2	2	1	1		1	8
54									1	1	1	1		4
57													1	1
Total	1	1	2	6	16	11	6	8	7	7	5	4	3	77

$r = +0.762 \pm 0.032$.

Aside from the high correlation in each of the two families, the almost exact correspondence of the two r's, the two η's, and the two Blakeman's tests is further evidence of a high order that there is a close correlation such as r can measure between spike density and awn length.

This correlation was also studied in another way. There was a series of F_3 progenies which were segregating for spike density. Since the awns on individual plants were measured it was possible to obtain the correlation between spike density × awn length within each progeny. Altogether, 13 such progenies chosen at random were correlated, with the results given in Table 18.

TABLE 18.—*Correlation coefficients* (r) *between spike density and awn length in 13 F_3 progenies, together with the probable errors* (*P. E.*) *and the ratio* $\frac{r}{\text{P. E.}}$

[Individual F_3 plants; grown in 1926 at Petersboro, Utah]

F_3 progeny	Correlation coefficient $r \pm$ P. E.	$\frac{r}{\text{P. E.}}$
15a-7-56	+0.3808±0.091	4.18
15a-7-61	+ .5735± .076	7.55
15a-7-69	+ .4396± .089	4.94
15a-7-75	+ .3325± .104	3.20
15a-7-82	+ .6726± .061	11.03
15a-7-87	+ .2291± .102	2.25
15a-7-93	+ .5351± .076	7.04
15a-7-105	+ .5803± .071	8.17
15a-7-116	+ .2986± .098	3.04
15a-7-124	+ .3695± .093	3.97
15a-7-132	+ .5882± .076	7.74
15a-7-140	+ .3701± .092	4.02
15a-7-145	+ .4490± .098	4.58

The r's vary from $+0.2291 \pm 0.102$ to $+0.6726 \pm 0.061$, and the ratio of $\frac{r}{\text{P. E.}}$ varies from 2.24 to 10.94. In only one case is r less than three times its probable error. In 5 out of the 13 cases, the r's are seven to eleven times their respective probable errors. In the other seven cases the ratio of $\frac{r}{\text{P. E.}}$ is between 3 and 5. All of these r's are plus, and all of them except one show odds of 25, or more, to 1 of being significant. In 9 of the 13 cases the odds are from 150 to infinity to 1 that the correlations are significant; 5 of the 9 have odds above 450,000 to 1.

The fact that all the correlation coefficients (r) are plus means that the longer the heads the longer the awns.

This evidence bears out strikingly the conclusion previously arrived at, largely by use of correlation ratios (η), that "between the two stable and definitely inherited characters of spike density and awn classes it seems reasonable to conclude that there is a strong suggestion of linkage between the factors for these two characters." [6]

Incidentally, also, here is evidence of the considerable importance of biometrical methods in analyzing genetic data. In the previous study the awns were grouped into four classes by inspection and the spike density was measured. The constant r is unadapted for correlation studies where one of the characters is grouped by categories

[6] STEWART, G. 1926. Op. cit.

rather than by actual discrete classes. The correlation ratio η, however, measures such correlations rather satisfactorily, and gave with sufficient clearness the indication that it was worth while to study the correlation between spike density and awn length with the care and the detail here reported.

In the correlations between spike density and height of plant, r is $+0.0696 \pm 0.049$ in family 15a–7; η is 0.3917 ± 0.042. In the other family, r is -0.6001 ± 0.049 and η is 0.7310 ± 0.036. In view of the contradiction between the two r's and in view of the fact that height of plant was markedly decreased by the poor and irregular soil on which some of the progenies of 15a–4 were grown, it does not seem wise to attach great importance to the r's themselves. On the other hand, both of the η's are significant in the light of their probable errors, being 9.4 and 20.4 times their respective probable errors. Of the two Blakeman's tests one is just less than 4 and the other just less than 3. Since in the previous study the η's were also large enough to suggest a correlation, and since all of the Blakeman's tests were likewise rather large, there seems to be at least a moderate suggestion of the presence of a correlation of some sort between spike density and height of plant. Since the r's were all very small in the previous study, and since in this study one is small and the other is known to be unreliable, there seems to be very little correlation such as r can measure. Repetition of the cross, with the F_3 progenies grown in careful replications and checked by being grown adjacent to the parental strains, would be necessary in order to measure this correlation. Sufficient refinement in the planting plan to permit an approximation of the variability due to soil heterogeneity would also be necessary.

When spike density is correlated with the number of spikelets to the spike, the two r's are -0.3169 ± 0.044 and -0.2447 ± 0.072, and the two η's are 0.4676 ± 0.039 and 0.3406 ± 0.068. The r's are 7.1 and 3.4 times their probable errors, and the η's 12.1 and 5 times their respective probable errors. The figures for the two Blakeman's tests are 3.4 and 1.5 for the two cases. There seems to be a suggestion of correlation between spike density and the number of spikelets, as indicated both by r and by η.

These two plant characters were not correlated in the previous study. Careful planning of an experiment should permit the measurement of this correlation.

The two r's between spike density × number of culms to the plant are so small that they should not be given much importance. The η's, however, are proportionately larger (0.3996 ± 0.041 and 0.3259 ± 0.069), which are 9.7 and 4.7 times their probable errors. No earlier correlations were made between these two characters.

Awn length correlated with height of plant gave one very small r and one fairly large one which is thought in this case (family 15a–4) to be the result of the poor development in height due to poor soil. It is probable that any study involving height of plant in family 15a–4 is unreliable. The correlations obtained, therefore, should not be considered significant, unless the η for family 15a–7 ($\eta = 0.2044 \pm 0.047$), which is reliable, should be so considered. This constant is 4.3 times its probable error. There was no other study to substantiate this suggestion of a correlation, as even the η's in the previous study for awn classes × plant height were small. Studies for awn

classes have only a suggestive bearing on measured awn-length relationships.

The correlation coefficients (r) for awn length × number of spikelets are negligible, being -0.0629 ± 0.049 and -0.016 ± 0.077. The η's are 0.2037 ± 0.047 and 0.3185 ± 0.069, both a little more than four times their probable errors. Since there were no additional studies to corroborate these correlations, no definite conclusions can be drawn without further study.

The two r's for awn length × number of culms are small both in absolute size and in relation to their probable errors. The η's are considerably larger (0.3586 ± 0.043 and 0.2982 ± 0.070), one being 8.3 and the other 4.3 times their probable errors. These four rather uniform results might be considered at least to suggest an important relationship. This is, however, decreased by the failure to establish definite evidence of inherited differences between the two parental strains in the number of culms to the plant.

Height of plant × number of spikelets and height of plants × number of culms both yielded r's and η's which on the surface would seem to be suggestive. These constants are all fairly large and are from 4.8 to 11.1 times their probable errors, except in one case which is 2.3 times its probable error. The fact that there may be a general tendency for plant height and number of culms to respond in the same way to environmental conditions weakens the value of these constants. It is elementary information that both of these two characters are extremely variable in the small cereals. They might even be physiologically correlated—that is, due to the same genetic factor. The case is not so clear in the correlation of height of plant × number of spikelets, for the number of spikelets is not nearly so variable as the height or the stooling characters. Carefully replicated plantings of good genetic material might reveal correlations between height of plant and number of spikelets. The r's in this case are $+0.2273 \pm 0.047$ and $+0.4344 \pm 0.063$, and the η's are 0.4078 ± 0.041 and 0.5025 ± 0.057. The ratios of $\frac{r}{\text{P. E.}}$ are 4.8 and 7, while the ratios of $\frac{\eta}{\text{P. E.}}$ are 9.9 and 8.7. These probably suggest real correlations, but would require much good statistical work to establish.

The study of number of spikelets × number of culms gave r's of $+0.1950 \pm 0.048$ and $+0.2517 \pm 0.072$, which are 4.1 and 3.5 times the probable errors. The η's are of about the same order (0.2435 ± 0.047 and 0.3195 ± 0.069), being 5.2 and 4.6 times their respective probable errors. Both Blakeman's tests are small and indicate that whatsoever correlation exists between these two plant characters is linear.

Partial and Multiple Correlations

In order to learn more nearly the true relationship between the various plant characters, partial correlations were computed. Partial correlation gives the degree of relationship between any two characters when the other variables are held constant. In these calculations the plant characters are numbered as follows:

(1) Spike density, (2) awn length, (3) height of plant, (4) number of spikelets per spike, and (5) number of culms per plant.

Partial correlations between spike density and awn length is designated as $r_{12.345}$, which means that the true relationship between characters No. 1 (spike density) and No. 2 (awn length) is obtained by holding constant the other three variables, 3, 4, and 5. The designation $r_{24.135}$ means that the true relationship between characters No. 2 (awn length) and No. 4 (number of spikelets) is found by holding constant characters 1, 3, and 5. Ten combinations of partial correlations are possible. These are shown in Table 19 beside the product-moment correlation coefficients (r) already given. Since family 15a–4 had been disturbed environmentally, partial correlations were calculated only for family 15a–7.

TABLE 19.—*Simple product-moment correlation coefficients* (r) *and comparative partial correlations between five plant characters in the wheat cross Kanred × Sevier*

[Family 15a-7; on means of F_3 progenies, grown in 1926 at Petersboro, Utah]

Product-moment correlations		Partial correlations	
Characters correlated	Correlation coefficients	Partial correlations	Correlation coefficients
Spike density × awn length	+0.6737±0.027	$r_{12.345}$	+0.6500±0.028
Spike density × plant height	+.0696±.049	$r_{13.245}$	+.0957±.049
Spike density × number of spikelets	−.3169±.044	$r_{14.235}$	−.3796±.042
Spike density × number of culms	+.1238±.049	$r_{15.234}$	+.0833±.049
Awn length × plant height	+.0985±.049	$r_{23.145}$	+.0045±.049
Awn length × number of spikelets	−.0629±.049	$r_{24.135}$	+.1737±.048
Awn length × number of culms	+.1611±.048	$r_{25.134}$	+.0625±.049
Plant height × number of spikelets	+.2273±.047	$r_{34.125}$	+.1997±.047
Plant height × number of culms	+.3065±.045	$r_{35.124}$	+.2488±.046
Number of spikelets × number of culms	+.1950±.048	$r_{45.123}$	+.1643±.048

The partial correlations and the simple product-moment correlations show important size in the same places. The simple product-moment correlation between awn length and number of spikelets was -0.0629 ± 0.049, whereas the partial correlations increased this to $+0.1737 \pm 0.048$, indicating that the true correlation was somewhat higher than was shown by the product-moment coefficient (r). There was no other material increase or decrease in size in any of the partial correlation coefficients as compared with the same respective product-moment coefficients.

Especially worthy of notice is the partial correlation for spike density × awn length when plant height, number of spikelets, and number of culms are held constant ($r_{12.345}$) $= +0.6500 \pm 0.028$). This also corroborates the previous conclusion that there is a real correlation between spike density and awn length. The partial correlation of $r_{14.235} = -0.3796 \pm 0.042$ gives a fairly strong correlation between spike density and number of spikelets when awn length, plant height, and number of culms are held constant.

A partial correlation coefficient of $+0.1997 \pm 0.047$ for plant height × number of spikelets and of $+0.1643 \pm 0.048$ for number of spikelets × number of culms suggest some, though probably not high, correlations. The tendency of plant height and number of culms to keep together is again apparent, but perhaps has also again to be discounted due to the strong positive influence of favorable soil conditions on both plant characters.

Multiple correlations were calculated for each of the five plant characters in relation to all of the other four characters—that is, the total effect of the other four characters on spike density is indicated by $R_{1.2345}=0.7333$. The actual value of this figure can be obtained in percentage from the equation v (per cent) $=100(1-\sqrt{1-r_2})$. When this calculation is made, it is found that 32 is the solution value obtained. Only 32 per cent, therefore, of the total variation in spike density is accounted for by the variation in the other four characters. This leaves 68 per cent of the variation to be explained by causes other than the ones here studied. Soil heterogeneity could not be determined from the data taken in this study. General observation of the soil used, in comparison with soils on which heterogeneity had been measured, would indicate it to be rather high in this experiment. The soil of the experimental field used was somewhat less uniform than another on which 15 per cent variability has been found. A brief summary of the multiple correlations for each character in relation to the other four is given in Table 20.

TABLE 20.—*Multiple correlations showing the total effect of the variability caused by the other four plant characters on the respective one indicated, in the wheat cross Kanred×Sevier*

[Family 15a–7; means of F_3 progenies, grown in 1926 at Petersboro, Utah]

Plant character	Multiple correlation	Correlation coefficient	Percentage of total variability accounted for
Spike density	$R_{1.2345}$	0.7333	32.0
Awn length	$R_{2.1345}$	.6591	25.4
Plant height	$R_{3.1245}$	.3784	7.5
Number of spikelets	$R_{4.1235}$	.4700	11.8
Number of culms	$R_{5.1234}$	.3647	6.9

As already noted, 32 per cent of the variation in spike density is accounted for in the variation of the other four characters studied. In the case of awn length 25.4 per cent of the variation is cared for, leaving 74.6 per cent due to unmeasured causes. In the other three cases, only 7.5, 11.8, and 6.9 per cent of the variation are accounted for by causes here studied.

Apparently only spike density and awn length account for any appreciable amount of the variation in the other one of this pair of characters. Even though far from complete in their influence, correlations which account for 32 and 25 per cent of the total variation must be ranked as important.

SUMMARY

This study is the outgrowth of a previous paper [7] in which the presence of a correlation was indicated between awn classes and spike density. In the previous study, a cross of Sevier×Federation, plants were grouped into four classes according to their expression of awns. There was a wide range in spike density in the F_3 progenies.

[7] STEWART, G. 1926. Op. cit.

Correlation ratios indicated considerable correlation between the measured spike densities and the awn classes.

The material used in the present study, a cross of Kanred × Sevier, was at that time being grown as part of an economic plant-breeding project. The fact that both parents were fully awned would have precluded any study of the awn character had not the hybrid progenies proved to be most desirable genetic material with which to study the correlation between spike density and awn length. There was a wide transgressive segregation in spike density and this seemed to be accompanied by differential length of awns. Accordingly, F_3 progenies seeded as a means of obtaining pure lines for rod-row yield tests were studied genetically.

The contrasting characteristics in the parental strains were as follows:

Kanred	*Sevier*
Spike density lax.	Spike density intermediate.
Fully awned.	Fully awned (awns shorter than those of Kanred).
Culms intermediate.	Culms long.
Kernels red.	Kernels white.
Glumes white.	Glumes bronze.

No exact data were taken in F_1, and in F_2 only color of grain was observed with care. Since all F_2 plants had been continued in F_3 progenies of 30 to 40 spaced plants each, these were studied, and the breeding behavior of each progeny taken as the true genotype of the F_2 plant from which it was descended. Each figure in the tables reported represents, therefore, a result obtained from studying each of the 30 or 40 plants in the F_3 progeny. Interspersed among the hybrids as they grew in the field were 8 rows of Sevier and 10 rows of Kanred. These were studied in the same fashion as the hybrids, each row being regarded as a unit. Three or four times as many parent rows would have been desirable. Of the hybrid progenies there were 187 in family 15a–7 and 77 in family 15a–4.

Spike density measurements of 10 central spikelet internodes were taken in millimeters on one leading spike of each plant in each progeny. The length of awns on the same spike was also measured in millimeters. The plant height was taken in centimeters to the base of the spike on the longest culm. After counting the number of culms on each plant and the number of spikelets on the spike measured for density, the worker who took the data classified each plant as to grain color and glume color.

INHERITANCE OF CHARACTERS

The F_2 genotypes as classified by their F_3 breeding behavior gave a close approximation of a 1:2:1 ratio for dense, heterozygous, and lax spikes. The dense-spiked and the lax-spiked progenies were true breeding. This was indicated by their coefficients of variability, which were approximately 10 per cent. That the progenies of intermediate spike density all segregated was indicated by coefficients of variability of 25 to 40 per cent. The numbers of homozygous dense, heterozygous, and homozygous lax progenies were, respectively, 45, 102, and 39 in one family, and 20, 36, and 21 in a second family, both of which are excellent fits for a 1:2:1 ratio.

In each of the three major groups of hybrids there was, however, a series of differences between various progenies. Some true-breeding dense progenies had considerably more dense spikes than did others. This was equally true of the heterozygous and of the lax progenies as well. The coefficients of variability for the means of the spike density of the hybrid progenies were about twice as great as for the parental rows. This is good evidence of the presence of minor factors in spike density inheritance. These minor factors were of such a nature as to prevent the definite recovery of the spike density of Sevier in a single true-breeding F_3 progeny. Only one of the 263 progenies studied even approached the Sevier parent in head density. There was a difference between the mean of the progeny and the mean of the most dense row of Sevier of about 1.5 times the probable error.

Kanred was distinctly shorter than Sevier. Some progenies were obtained, the mean height of which was somewhat greater than that of either parent, though some Kanred rows were shorter than any of the hybrid progenies. The total range in height of the progenies was about equal to the range covered by the two parents. Though segregation in height was clearly manifest, no ratios were found which indicated the nature of the segregation. No definite conclusions were reached as to the inheritance of the number of spikelets or the number of culms.

The inheritance of grain color gave a close fit between the expected and the observed on the basis of a three-factor difference. In F_3 and F_4, true-breeding red-grained progenies and true-breeding white-grained progenies were obtained, as well as others segregating 63:1, 15:1, and 3:1 for red and white grain.

A ratio of 3 bronze-chaffed progenies to 1 white-chaffed was obtained

CORRELATION STUDIES

Simple, partial, and multiple correlations were calculated in all possible combinations between (1) spike density, (2) awn length, (3) height of plant, (4) number of spikelets, and (5) number of culms.

Spike density and awn length gave high correlations. In two families r was $+0.674 \pm 0.027$ and $+0.762 \pm 0.32$, which are 25 and 23.6 times their probable errors. In addition, 13 progenies segregating for spike density gave r's which varied from $+0.229 \pm 0.102$ to $+0.673 \pm 0.061$. In 12 of these 13 correlations, r is more than 3 times its probable error and in 5 cases from 7 to 11 times the probable error. Every r between spike density and awn length was plus, indicating that the longer spikes had longer awns. The partial correlation was also high, being $+0.6500 \pm 0.028$ when height of plant, number of spikelets, and number of culms were held constant. In the multiple correlations for spike density and awn length, R equaled 0.733 and 0.659, respectively.

There seems no doubt, therefore, that there is linkage between spike density and awn length. There is also a suggestion of correlation between spike density and number of spikelets and between height of plant and number of spikelets.

A COMPARISON OF SOME STRAINS OF RHIZOCTONIA SOLANI IN CULTURE[1]

By John Monteith, Jr., *Associate Pathologist, Office of Vegetable and Forage Diseases, Bureau of Plant Industry, United States Department of Agriculture,* and Arnold S. Dahl, *Graduate Assistant, University of Wisconsin*

INTRODUCTION

In studying the large brown-patch disease of turf, described by Piper and Coe (*6*)[2] as due to *Rhizoctonia solani*, it was found that the causal organism varied in culture in several respects from some stock cultures of *R. solani*[3] obtained from potato tubers. There was found a marked difference in their pathogenicity on grass, and it seemed possible that they might be distinguished by certain variations in growth characteristics on artificial media. The investigations here reported were undertaken in an attempt to determine to what extent such characteristics would prove reliable in distinguishing the Rhizoctonia on grass from the potato organism, either as a distinct species or as a strain of *R. solani*. Several cultures were therefore obtained from different sources to compare with the grass fungus under various cultural conditions.

SOURCE OF STRAINS

Two isolations of the Rhizoctonia producing brown patch of grass were used in these comparisons. One of these (1) was obtained from diseased bent-grass leaves growing in the greenhouse at Madison, Wis., in the spring of 1924, and the other (2) was isolated in June of the same year from grass injured by brown patch in the turf garden on the Arlington Experiment Farm, Rosslyn, Va. Since the turf growing in the greenhouse at Madison was originally sent from the Arlington turf garden, these two cultures were probably of the same origin.

Cultures 3 and 4 were obtained from B. L. Richards, isolated from potato and identified by him as *Corticium vagum* B. and C. These two represent his cultures Nos. 35 and 131, respectively. Cultures 5 and 6 were isolated by Freeman Weiss in Washington from diseased potato tubers during the summer of 1924. Culture 7 was isolated from a sclerotium on a potato tuber at Madison in January, 1925. Culture 8 was from a stock culture in the laboratory of plant pathology at Madison, Wis. This represents the culture known as Rosenbaum's strain R5, isolated in 1916 in Maine from a diseased potato stem. Culture 9 was isolated from diseased peas in the spring of 1924 by F. R. Jones in Wisconsin.

[1] Received for publication Dec. 1, 1927; issued July, 1928. Work done in the department of plant pathology, University of Wisconsin, in cooperation with the United States Golf Association Green Section.

[2] Reference is made by number (italic) to "Literature cited," p. 903.

[3] No attempt will be made to make detailed references to the extensive literature dealing with this fungus. The writers are aware of the divergence of opinion as to what constitutes a "species" or "strain" of Rhizoctonia, as expressed in such publications as those by Duggar (*2*), Peltier (*5*), Matz (*4*), Matsumoto (*3*), Briton-Jones (*1*), and Thomas (*7*).

METHODS

Differences between the fungi isolated from grass and those from potato proved to be practically the same on several culture media, so it was decided to confine the study chiefly to a single medium. Potato-dextrose agar was chosen for this purpose, since it proved to be favorable for all the cultures, besides having several other desirable features. This was prepared in the usual manner, a decoction of 200 gm. of potato per liter of the medium with 2 per cent dextrose and 1½ per cent agar being used. In pouring plates sufficient agar was used to make a layer about 5 mm. thick. This provided a sufficient reserve of moisture to take care of any small loss due to evaporation in cultures grown for several days. Tests showed that there were decided differences in initial growth, depending on whether transfers were made from old or from young cultures. In some cases inoculations made with the tips of growing mycelium had entirely crossed the plate before similar inoculations made from old cultures had started to develop. Therefore all further inoculations were made by transferring pieces of agar containing tips of growing mycelium taken from the borders of young cultures.

INFLUENCE OF TEMPERATURE ON RATE OF GROWTH

The influence of temperature on the rate of growth was studied in incubators maintained at 10°, 15°, 20,° 25°, 30°, and 35° C. In the preliminary tests, when Petri-dish cultures were piled together in one of the temperature chambers, there was frequently a noticeable difference in growth in the dishes in the center of the pile compared with those at the top or bottom. These differences were most marked in chambers where the temperatures were well above or below that of the laboratory and apparently were dependent on the time required for the plates to reach a temperature equilibrium. Therefore, in subsequent work the medium was poured and the dishes were kept in their respective incubators for several hours to bring them to the desired temperature before inoculating. Transfers were made and later observations were recorded as rapidly as possible, to avoid any serious temperature change while the material was out of the incubator.

As the work progressed it was found that at a given temperature a more vigorous growth frequently occurred if the transfer was made from a culture grown at this same temperature than if the transfer was made from a culture grown at some other temperature. Thus, if two cultures were kept at 15°, one inoculated from a culture grown at 15° and the other from one at 25°, the transfer from the 15° plate would make a much more rapid growth. This behavior was more marked in the case of certain cultures than in others. To avoid this difference, therefore, inoculations for any temperature were made from a plate previously kept at that same temperature.

When transfers were made from the same plate and even from corresponding segments of mycelium there was some noticeable difference in the rapidity with which the new cultures started growth, although when once established they grew at very nearly the same rate. To avoid this initial difference, measurements were made about 24 hours after transferring, at which time new growth had

started in most cases. Readings were again made when the mycelium had reached the edge of the plate at the optimum temperature. From these measurements the rate of radial growth per hour during this interval was calculated. Figure 1 shows the average growth for a typical temperature series, based on these calculations.

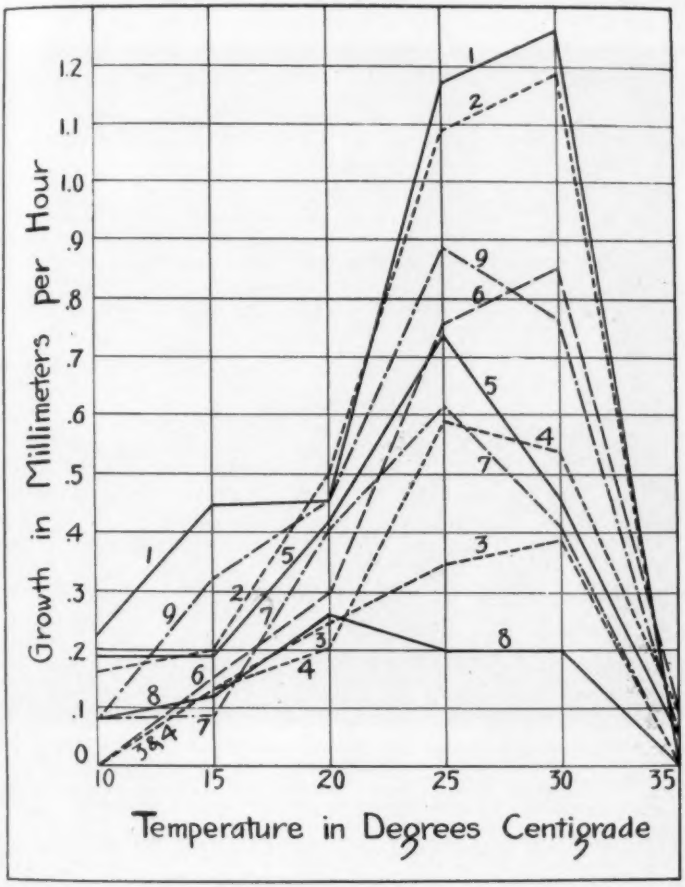

FIG. 1.—Effect of temperature on rate of growth of nine cultures of *Rhizoctonia solani* on potato-dextrose agar

It will be seen that in general the optimum temperature for growth was 25° or 30°. The two grass strains (1 and 2) showed a similar response to temperature, as might be expected, since their origin was similar. At 25° and 30° these cultures were much more vigorous than any of the potato strains. The difference between them and certain potato strains, however, was not so great as the difference existing between various strains isolated from potato tubers. In

comparing the curves it is seen that the cultures which had been kept on artificial media for the longest periods, namely, 3, 4, and 8, grew less vigorously than some of the more recent isolations. This might indicate a decrease in vigor due to continued artificial culture. However, it will be noted that the fungus representing the shortest period in culture, No. 7, was also one of the less vigorous group.

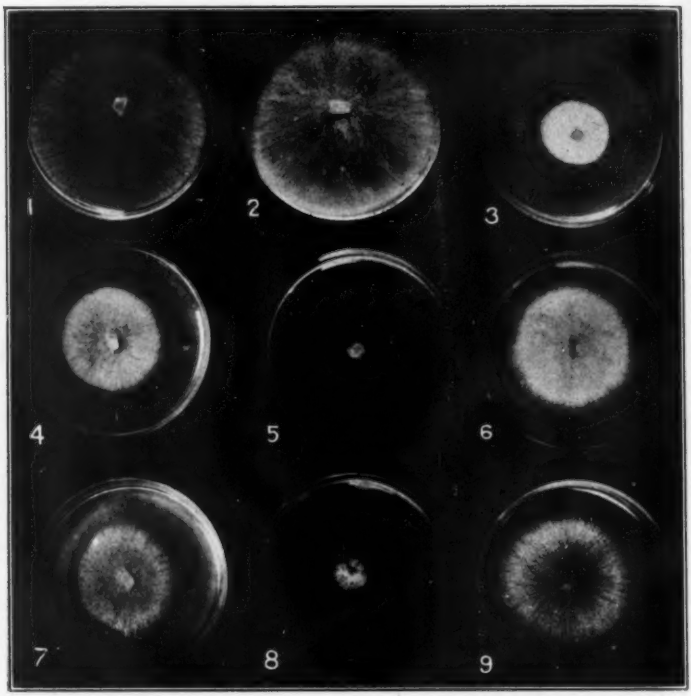

FIG. 2.—Nine cultures of *Rhizoctonia solani* grown on potato-dextrose agar at 25° C. Cultures 1 and 2 were isolated from grass, and the others were obtained from potatoes from different localities. The two from grass could readily be distinguished from the others at this temperature, but, owing to the wide variation shown in the potato strains, such differences would probably be of little value in distinguishing strains if a large collection were studied

INFLUENCE OF TEMPERATURE ON CHARACTER OF GROWTH

When the cultures were several days old there were striking differences in the appearance of the colonies at different temperatures. An attempt was made to distinguish strains based on the character of growth. For this purpose such characters as color of mycelium or sclerotia, discoloration of medium, amount and type of aerial growth, shape and size of sclerotia, and various other such characters were used.

From these observations it was evident that certain cultures could be distinguished readily from others. Figure 2 shows a group of

typical colonies of the same age grown at 25° C. This illustrates the close similarity between the two grass cultures, 1 and 2. These are readily distinguished from such potato cultures as 3 and 6. The differences in these cases, however, are no more marked than are the differences between such strains as 5 and 6, which were both isolated from potato tubers at about the same time. These macroscopic differences were furthermore complicated by changes at other temperatures; for instance, cultures 5 and 7 had very much the same appearance at 25°, but at some of the other temperatures they could be readily distinguished by their growth characters. In some cases there was a striking difference in type of growth with a change of only a few degrees of temperature. Figure 3 shows such a difference in the case of culture 6, which has a decidedly different type of growth

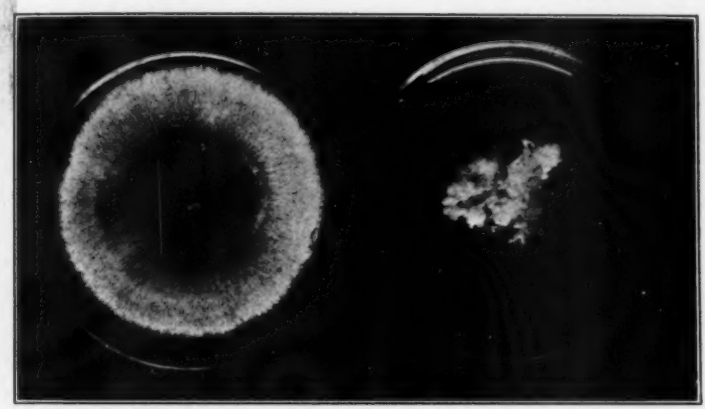

FIG. 3.—Strain No. 6, grown at 33° (left) and 35° C. (right) on potato-dextrose agar. The 33° culture was 3 days old, whereas the 35° culture had been growing 7 days

at 33° and at 35°. In other cases, as in the two grass cultures, the growth characters were sufficiently constant throughout the temperature range to enable one to distinguish them from the other cultures.

The extent to which media are discolored is a characteristic sometimes used in distinguishing strains of Rhizoctonia. Nos. 1 and 2, from grass, did not noticeably darken the agar at any temperature. No. 8 (Rosenbaum's R5 strain) darkened agar very markedly at 20°, 25°, and 30°, but caused no discoloration at 15° C. Cultures 6 and 9 did not blacken the agar at any temperature except 35°, but there the discoloration was very distinct.

Cultures 1, 6, and 7 were grown for three months in flasks containing a mixture of corn meal and sand. These were kept at approximately 15°, 25°, and 30° C. Cultures 1 and 7 produced large compact sclerotia at 15° and much smaller and less compact masses at 30°. Culture 6, on the other hand, did not produce any at 15°, but produced many small sclerotia at 30° C. Observations on color as well as on type of sclerotia failed to reveal any reliable means for distinguishing the grass strain from the fungus found on potato.

INFLUENCE OF TEMPERATURE ON DIAMETERS OF HYPHAE

To determine the extent to which the diameters of the hyphae might be used as a guide to distinguish strains, a series of measurements was made on three cultures growing at 15°, 20°, 25°, and 30° C. When these were 2 days old, cover glasses were placed over the outer edges of the colonies and measurements were made near the tips of the new hyphae, a point just back of the origin of the most recent lateral branch being selected. One hundred such measurements were made on duplicate plates for each temperature. The averages of these measurements are given in Figure 4, which shows the variation found in the diameters of the hyphae grown at different temperatures. It will be noted that the grass culture tended to increase rapidly in size with increase in temperature. This corresponds fairly closely with the increased radial growth illustrated in Figure 1. On the other hand, the other two from potatoes in general showed a tendency toward a decrease in diameter of hyphae with increase in temperature. These differences, however, are comparatively slight. A comparison of the diameter of growth at 15° and 30°, especially in the cases of 1 and 5, indicates how confusing it may be to use diameters of hyphae as a characteristic for distinguishing strains if the temperature factor is not considered.

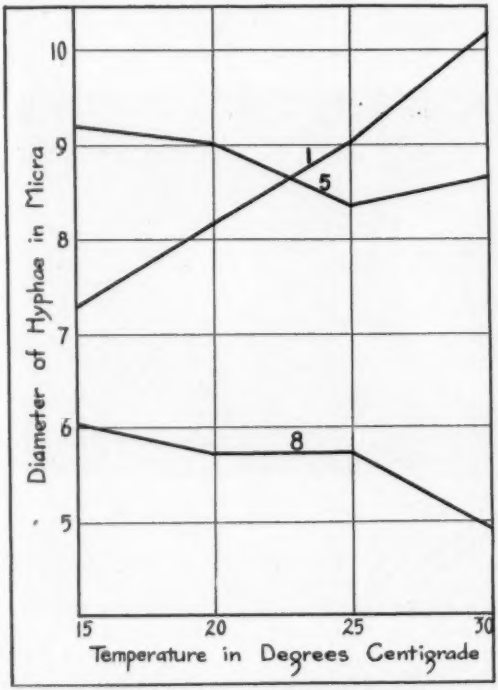

FIG. 4.—Variations in diameters of terminal hyphae in three strains of *Rhizoctonia solani* grown on potato-dextrose agar at different temperatures

INFLUENCE OF ACIDITY OF MEDIUM ON GROWTH

Rhizoctonia is apparently able to grow over a wide range in degree of acidity or alkalinity. Variations in acidity of the culture medium, as expressed on the P_H basis, produced marked variations in the growth character of different cultures. Thus, color variations, from hyaline to dark brown, were observed in mycelium, the darker color occurring usually near the neutral point. The type and color of sclerotia in each culture also showed marked variations due to degree of acidity. These differences, however, were not confined to, nor

sufficiently correlated with, any one culture or group of cultures in a way to justify their use as a key to differentiating strains.

SUMMARY AND CONCLUSION

In comparing on media cultures of Rhizoctonia isolated from diseased grass, numerous minor differences were found between them and cultures of this fungus isolated from potato. An attempt was made to determine to what extent these differences could be used to define the relationship of the Rhizoctonia found on grass to that obtained from potato or other hosts. Cultures of *Rhizoctonia solani* from different sources were therefore compared on several media under various conditions of temperature and acidity.

Using as a criterion various macroscopic and microscopic characteristics of growth such as have been used at times to define strains or even "species" of Rhizoctonia, it was found that temperature and acidity of the medium frequently so modified these characters that they appeared unreliable as dividing lines between species or even "strains" unless much detailed definition of certain environmental factors be included. Two cultures isolated from grass under most of the conditions studied could be distinguished from all other cultures of Rhizoctonia under observation. However, since each culture isolated from potato gave a somewhat different response, it is quite probable that if a sufficient number had been included there would have been found gradations covering the whole range between any of the extremes observed in this work.

The most outstanding distinction found in the grass fungus was its more rapid growth, especially at 25° and 30° C. This, however, is more likely to be a coincidence than an actual difference existing between all Rhizoctonia on grass and on potato.

From this study it is therefore concluded that the morphological and physiological differences observed between the Rhizoctonia causing large brown-patch disease on grass and stock cultures of *Rhizoctonia solani* are not sufficiently fixed and definite to serve as a reliable means for separating it from the variable fungus found on potato and other plants which is at present included in the species *R. solani*.

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DIFFERENCES IN RESISTANCE TO BACTERIAL WILT IN INBRED STRAINS AND CROSSES OF DENT CORN[1]

By C. S. REDDY, formerly *Associate Pathologist*, and J. R. HOLBERT, *Agronomist*, *Office of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

Bacterial wilt or Stewart's disease of sweet corn, caused by *Aplanobacter stewarti* (E. F. S.) McC., has been known for more than 30 years (*11*).[2] Further studies have added to the knowledge of its host range, geographical distribution, methods of dissemination, and economic importance (*1*, *3*, *4*, *5*, *8*, *9*, *10*). Many varieties of sweet corn have been tested to determine their relative susceptibility to this disease (*2*, *3*), and the value of seed treatment for its control has been investigated (*3*, *7*); yet little has been accomplished in reducing the annual losses. In fact, the losses have tended to increase because of the commercial practice of growing early-maturing, highly susceptible sweet-corn varieties, such as Golden Bantam, in States where the disease is prevalent. The short-season sweet-corn varieties, as a group, are susceptible to bacterial wilt. It is probable, therefore, that if late planting becomes necessary in corn-borer control the wilt problem will be accentuated.

It is known that the prevalence of bacterial wilt of sweet corn decreases from south to north in the United States. The disease is seldom found very far north of the Corn Belt proper. From the standpoint of resistance and susceptibility, the late-maturing varieties of sweet corn are comparatively resistant and the early maturing varieties are susceptible. Dent corn has been found to be more resistant than sweet or flint corn (*5*). If a few exceptional years are left out of consideration, it may be said that losses from this disease in late varieties of sweet corn and in dent corn are of minor importance.

From the above-stated facts the control of bacterial wilt would seem to be simple; that is, (1) in the Corn Belt, where the disease occurs, grow the late-maturing varieties, which are resistant; and (2) north of where the disease occurs, grow the short-season, susceptible varieties, if desired. Although corn-canning companies could give greater consideration to these facts than they do at present, the market gardeners and home gardeners are limited to their localities and prefer to grow the popular varieties whenever they can. The high-quality varieties for table use on the ear, in general, are early maturing. At present no such high-quality, early-maturing variety of sweet corn is known to be wilt resistant. While the preliminary investigations here reported were conducted with dent corn, the results suggest the possibility of developing wilt-resistant sweet-corn varieties of high quality by means of the pure-line method of breeding.

[1] Received for publication Mar. 2, 1928; issued July, 1928. These investigations were conducted near Bloomington, Ill., in cooperation with Funk Bros. Seed Co.

[2] Reference is made by number (italic) to "Literature cited," p. 910.

MATERIAL AND METHODS

Certain field experiments were undertaken in 1926 with a view to determining the relative resistance to bacterial wilt of a number of inbred lines and crosses of yellow dent corn, grown for another purpose. These experiments were conducted near Bloomington, Ill., on uniform, well-drained, productive brown silt loam soil. Two series of experimental plots, A and B, were located about 1 mile apart on adjoining farms where the soil conditions were similar.

In series A the corn was planted during the first week in May and in series B it was planted during the last week in May. The individual plots in series A included 45 plants, 15 of which were inoculated and 30 uninoculated. The individual plots in series B included 72 plants, 24 of which were inoculated and 48 uninoculated. No attempt was made to thin to a uniform stand either before or after inoculation.

The seed used included several inbred strains of yellow dent corn and their recombinations. The number of years of inbreeding ranged from five to nine. All strains matured in approximately the same number of days.

The organism used in the inoculations was isolated about four weeks preceding its use, from young Golden Bantam sweet-corn plants grown in Virginia in 1926.

The inoculations were made by means of a hypodermic syringe when the plants were from 24 to 30 inches high. Approximately 0.2 c. c. of a suspension of the bacteria in distilled water was injected into the central portion of each stalk about 2 inches above the crown of the plant. Previous experiments had shown that it was unnecessary to inject distilled water into the control plants (*6*). Yields of sound air-dried corn on the ear were used as the basis of judging the relative resistance of the different strains.

RESULTS

Data on the resistance to bacterial wilt of 15 inbred strains of yellow dent corn and of 4 recombinations are given in Table 1.

Data in Table 1 show that reductions in yield of inbred strains of yellow dent corn, following inoculation with *Aplanobacter stewarti*, varied in series A from 5.8 per cent to 67.3 per cent. Unfortunately the strains that proved to be very high in resistance to bacterial wilt in series A, the earlier planting, had not been included in series B, but the strains that were intermediate in resistance in series A were also intermediate in series B. Strain 1 was very susceptible in both series. The greater reduction of strain 10 in series B was due largely to the fact that the three progenies used included only one of the more resistant ones. The appearance of this highly resistant progeny of strain 10, in contrast to one of the susceptible progenies of strain 1, is illustrated in Figure 1. It will be noted from Figure 1, A, that strains 10 and 1 were approximately equal in vigor. In most cases first-generation crosses were more resistant than their component inbred strains. It is interesting to note that the recombinations of strains 10 and 13 showed no reduction following inoculation, whereas the recombinations of strains 15 and 13 showed a reduction of 24 per cent following inoculation. These two crosses were comparable both in vegetative vigor and in date of maturity.

FIG. 1.—Inbred strains of yellow dent corn, uninoculated and inoculated with *Aplanobacter stewarti:* A.—*a*, strain 10, uninoculated; *b*, strain 1, uninoculated. B.—*a*, strain 10, inoculated; *b*, strain 1, inoculated

TABLE 1.—*Differences in resistance to Aplanobacter stewarti of inbred strains in yellow dent corn, and crosses between them, as indicated by mean percentage reductions in yield of ear corn at Bloomington, Ill., in 1926, following artificial inoculation*

Strain Nos. or F_1 from cross	Number of years of inbreeding	Number of progenies		Mean percentage reduction in yield		Rank in resistance
		Series A	Series B	Series A	Series B	
10×13			1		0	1
14×10			2		0.4 (±3.1)	2
2	9	6		5.8 (±2.0)		3
3	9	9		9.2 (±3.6)		4
5	9	9		9.2 (±3.6)		4
8×9			6		10.6 (±1.9)	5
13	9	39		12.9 (±2.4)		6
10	6	11	*3	13.7 (±3.0)	28.2 (±16.2)	7
9	8		4		16.9 (±8.5)	8
4	9		5		20.7 (±2.0)	9
15×13			6		24.0 (±6.3)	10
6	9	7		24.0 (±5.7)		10
14	9	11		24.4 (±5.1)		11
12	5		22		26.0 (±1.6)	12
15	9		3		29.9 (±8.9)	13
11	9	7		38.8 (±4.4)		14
7	9		3		42.5 (±5.2)	15
8	9		5		51.6 (±8.9)	16
1	7	*4	9	67.3 (±8.0)	68.4 (±3.3)	17

* These progenies are included in those of the other series.

The data in Table 2 indicate that all the progenies of some inbred lines were very susceptible (strain 1), or resistant (strain 2), or uniformly intermediate (strain 4). Other strains, apparently homozygous for a number of visible plant characters, showed wide ranges in reaction to inoculation (strains 8, 13, and 14). However, in a strain as susceptible as strain 1 there was considerable variation in the extent of injury following inoculation. (Fig. 2.) The yield of progeny 1 of this strain was reduced 30 per cent by inoculation (fig. 2, B, *a*), but there was not a single sound ear in the inoculated portion of progeny 2 (fig. 2, B, *b*).

TABLE 2.—*Differences in resistance to Aplanobacter stewarti in the various progenies in six inbred strains of yellow dent corn, as indicated by mean percentage reductions in yield of ear corn at Bloomington, Ill., in 1926, following artificial inoculation*

Strain No.	Number of progenies	Mean percentage reduction in yield	Rank in resistance	Percentages of progenies with percentage reductions of—					
				1–5	6–10	11–25	26–50	51–75	76–100
2	6	5.8 (±2.0)	1	33	33	33	0	0	0
13	39	12.9 (±2.4)	2	41	8	20	28	3	0
4	5	20.7 (±2.0)	3	0	0	80	20	0	0
14	11	24.4 (±5.1)	4	18	10	18	36	18	0
8	5	51.6 (±8.9)	5	0	20	0	20	40	20
1	13	68.1 (±3.1)	6	0	0	0	15	62	23

FIG. 2.—Progeny rows of inbred strain 1 of yellow dent corn, uninoculated and inoculated with *Aplanobacter stewarti:* A.—*a*, progeny 1, uninoculated; *b*, progeny 2, uninoculated. B.—*a*, progeny 1, inoculated; *b*, progeny 2, inoculated

SUMMARY

Bacterial wilt is economically important, especially on certain popular varieties of sweet corn.

The fact that the disease has been studied for 30 years without the losses being materially reduced indicates that no easy method of control is likely to be found.

Data are presented which show wide differences in reaction to inoculation with *Aplanobacter stewarti* in a number of inbred lines of yellow dent corn maturing in the same length of time. All the progenies of some inbred lines were uniformly high in resistance. These results suggest the possibility of developing resistance to bacterial wilt in some of the popular wilt-susceptible varieties of sweet corn. Other inbred lines, however, were less resistant and still others were uniformly susceptible.

There was no apparent correlation between resistance and vegetative vigor.

There was no apparent correlation between resistance to bacterial wilt and resistance to other important diseases, although strains with high wilt resistance usually were free from other diseases.

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THE HOST RELATIONSHIP OF THE TREMATODE GENUS ZYGOCOTYLE [1]

By EMMETT W. PRICE

Associate Parasitologist, Zoological Division, Bureau of Animal Industry, United States Department of Agriculture

HISTORICAL REVIEW

The occurrence of the same species of trematode in hosts as widely separate zoologically as birds and mammals is not common. A few such cases have been recorded for the Heterophyiidae, but so far as the writer is aware only one record exists in which an amphistome, normally a parasite of water birds, has been found in a mammal.

Diesing (*2*) [2] described an amphistome, *Amphistoma lunatum*, from the cecum of a ruminant, *Cervus dichotomus*, and also from the ceca of the following birds: *Anas melanotus*, *A. ipecutiri*, and *Himantopus wilsonii*. The specimens upon which his description was based had been collected several years previously by Natterer, in Brazil. Because of the wide difference in hosts, Dujardin (*4*) considered the record from *C. dichotomus* as probably a mistake, and later Diesing (*3*) apparently arrived at the same conclusion. Fischoeder (*5*) redescribed this species from some of the original material in the Vienna Museum, but was unable to find any essential differences between the mammalian and bird forms, and concluded that the citation of *C. dichotomus* as a host for this trematode was probably due to an error in labeling. Stunkard (*7*) transferred *A. lunatum* to the genus Zygocotyle, a genus proposed by him for a species of trematode, *Zygocotyle ceratosa*, found in the intestine of a duck, *Anas platyrhynchus*, from Nebraska.

Recently, through the courtesy of M. C. Hall, chief of the Zoological Division, the writer has been afforded an opportunity to study specimens of an amphistome reported by him (*6*) from the cecum of a cow, *Bos taurus*. These specimens were collected at Penonomé, Panama, June 10, 1926, and have been identified by the writer as *Zygocotyle lunata* (=*Amphistoma lunatum*). Aside from the fact that this constitutes a new host and geographical record for the species, it also apparently validates the previous doubtful record from *Cervus dichotomus*, and constitutes a second occurrence of this amphistome in a mammalian host.

The specimens, 30 in number, had been fixed in cold formaldehyde and many were much contracted. There is also considerable variation in size due to age, some being quite small and immature. The following description is based on 10 of the more mature specimens:

DESCRIPTION OF ZYGOCOTYLE LUNATA FROM THE COW

Length, 4 to 6 mm.; width, 2 to 3 mm. The body form is oval and strongly curved ventrally (fig. 1), this curving being present in live specimens and perhaps exaggerated by preservation in a cold fixative. The color is yellowish white and

[1] Received for publication Mar. 19, 1928; issued July, 1928.

[2] Reference is made by number (italic) to "Literature cited," p. 914.

the cuticle is devoid of spines. The oral sucker is subterminal, strongly muscular, and is 390 to 650 μ in diameter. The oral evaginations or pharyngeal pouches are thick walled, 172 to 250 μ long and 117 to 130 μ wide, and open into the oral cavity near the origin of the esophagus. The acetabulum is large, strongly muscular, and opens ventrally. The opening is slightly wider than long, measuring 0.78 to 1.1 mm. by 0.91 to 1.2 mm. The posterior part of the acetabulum is cup shaped and the anterior part is conical, the apex being directed anteriorly and dorsally; the posterior edge is provided with a characteristic flap, which terminates on each side in a small conelike projection. The esophagus is 325 to 455 μ long, and is provided distally with a strong, muscular bulb 190 to 416 μ long by 130 to 260 μ wide. The intestinal ceca are thick walled and terminate at the level of the acetabular opening or a short distance cephalad of the opening. The testes are elongated transversely and lobulated or irregular in outline; in some of the smaller specimens the edges are almost smooth. The anterior testis measures 325 to 650 μ by 260 to 390 μ. The posterior testis lies immediately caudad of the anterior testis and measures 520 to 650 μ by 325 to 520 μ. The seminal vesicle is coiled and lies in front of the anterior testis. Cirrus sac absent. The ovary is oval or slightly irregular in outline, 156 μ by 260 μ in diameter, and lies about midway between the anterior part of the acetabulum and the posterior border of the posterior testis. In fully mature specimens, the uterus extends anteriorly in close coils to the genital opening, which is situated immediately caudad of the intestinal bifurcation. The vitellaria are composed of large

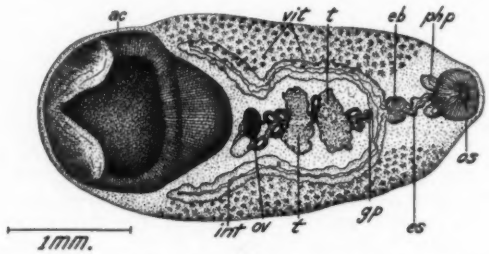

FIG. 1.—*Zygocotyle lunata*. Young specimen from cow; ventral view: *ac*, acetabulum; *gp*, genital pore; *int*, intestine; *eb*, esophageal bulb; *es*, esophagus; *os*, oral sucker; *or*, ovary; *php*, pharyngeal pouch; *t*, testis; *vit*, vitellaria

follicles lying extracecally and extending from the level of the posterior end of the pharyngeal pouches to the level of the opening of the acetabulum. The eggs are oval, operculated, yellowish in color, and 130 to 143 μ long by 77 to 90 μ wide.

COMPARISON WITH SPECIMENS OF ZYGOCOTYLE FROM BIRD HOSTS

In view of the fact that Stunkard (7) considers the amphistome of ducks in North America as distinct from *Zygocotyle lunata*, the writer has compared the form described in this paper with amphistomes in the helminthological collection of the Bureau of Animal Industry, which had been collected from several bird hosts.

The material for this comparison consisted of: Six specimens collected by B. Schwartz from the domestic goose, *Anser anser domesticus*, at Washington, D. C., February 3, 1919; 1 specimen collected by E. A. Chapin from the redhead duck, *Marila americana*, at Bush River, Md., January 17, 1924; 6 specimens collected by the writer from the green-winged teal, *Nettion carolinensis*, at Wellborn, Tex., November 15, 1921; and 1 specimen from the jacksnipe, *Gallinago delicata*, collected also by the writer at Wellborn, Tex., October 5, 1921.

TABLE 1.—*Comparative measurements of specimens of Zygocotyle from the cow with those from various bird hosts*

Name of host	Length of specimen	Width of specimen	Diameter of oral sucker	Pharyngeal pouch	Esophagus	Esophageal bulb	Anterior testis	Posterior testis	Ovary	Egg	Acetabulum
	Mm.	*Mm.*	μ	μ	μ	μ	μ	μ	μ	μ	*Mm.*
Bos taurus	4–6	2–3	390–650	172–250× 117–130	325–455	190–416× 130–260	325–650× 260–390	520–650× 325–520	175–260	130–143× 77–90	0. 78–1. 1× . 91–1. 2
Anser anser domesticus	4–6	2–3	390–520	156–260× 130–195	455–650	195–156	680–900× 325–520	455–910× 286–520	175–325× 175–365	128–153× 76–96	. 58–. 65× . 91–1. 2
Marila americana	8	3	455	221×156	650	225×175	* 780	(*)	390×225	140×96	. 78×. 91
Nettion carolinensis	3–7	1. 5–4	286–520	130–260× 65–208	260–520	167–260× 156–200	364–650× 221–520	390–585× 234–588	143–221× 71–195	124–150× 75–96	. 72–. 78× . 6–. 95
Gallinago delicata	3	1. 5	286	130×65	260	169×156	364–221	390×234	143×91	125×75	. 55×. 52
Zygocotyle ceratosa (from Stunkard, 1917)	3–6	1. 45–2. 4	370–530	150–220× 70–100	50–370	200–450× 180–230	200–300	530–780	200–500	140×83	1. 1×. 74
Zygocotyle lunata from (Fischoeder, 1903)	5–9	1. 6–3	.....	300–350× 100–120	1, 000–1, 300	500×400	500	.....	300	145–150× 72–75	. 8–1×1–1. 5

* Obscured by the greatly distended uterus.

Observations on these specimens showed them to agree in all essential characters, and they are therefore considered to be of the same species. Considerable variation was found in the measurements, but as there was no great disparity in the shape and relative position of the different organs, these differences are not regarded as significant, especially since as great variation was found in specimens from the same host as in those from the different hosts. A rather remarkable difference was observed in the egg sizes of the different specimens, the variation being in some instances as much as 25 μ in the length and 21 μ in the width. These differences are similar to those reported by Cort (*1*) for other trematodes.

Table 1 gives a comparison of the measurements obtained, and for further comparison the measurements given by Stunkard (*7*) for *Zygocotyle ceratosa* and by Fischoeder (*5*) for *Z. lunata* are included.

It will be noted in this table that for the most part the measurements of the specimens studied by the writer intergrade with those given for *Zygocotyle ceratosa* and *Z. lunata*. In view of this fact, and in the absence of consistent morphological differences, it is the opinion of the writer that the form occurring in ruminants is the same as that occurring in birds, and that the recognition of *Z. ceratosa* as a distinct species is not justified. For the time being *Z. ceratosa* is regarded as a synonym of *Z. lunata*.

SUMMARY

A species of amphistome, *Zygocotyle lunata*, apparently normally parasitic in water birds, is reported for the first time in a domestic ruminant, *Bos taurus*. This record is the second report of this species from ruminants, the first being from a deer, *Cervus dichotomus*.

The following new bird hosts are reported for this trematode: *Anser anser domesticus*, *Gallinago delicata*, *Marila americana*, and *Nettion carolinensis*.

A comparison of specimens from the cow with specimens from water birds indicates that they are specifically identical, and that the recognition of *Zygocotyle ceratosa* as a distinct species is not justified. *Z. ceratosa* is, therefore, regarded as a synonym of *Z. lunata*.

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